PHYSIOLOGY

Challenges and the Way Forward

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Challenges and the Way Forward

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Physiology
Challenges and the Way Forward

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Physiology – Challenges and the Way Forward

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IUPS Foreword

This second global report, *Physiology: Challenges and the Way Forward*, was prepared by the capable and skillful hands of Professors Jayasree Sengupta and Susan M. Barman, Chair and Vice Chair of the Board of the General Assembly (BGA), during the most difficult time faced by humankind in modern history because of the unprecedented raging and devastating COVID-19 pandemic. Despite the imposing hardship in all walks of life worldwide, I do find that the successful launching of this 2022 BGA Report shines a light in the dark that solidifies our belief that IUPS plays an important role in global science in general and physiology in particular.

IUPS is a collective of national physiological societies and federations worldwide. Its commanding position in the international physiological family is highlighted by the sheer demonstration of kinship when 36 national societies representing physiologists from the Americas, Europe, Africa, Asia, and Oceania returned their responses to questionnaire requested by the BGA. The collective inputs that form the basis of this report therefore represent a true global perspective on the current status and future goals of physiology research and teaching.

In the first report on Physiological Sciences presented by the BGA, *Physiology Current Trends and Future Challenges,* seven recommendations were proposed to strengthen global physiology. From the detailed narratives in this report, it is apparent that these recommendations have been or are beginning to be implemented. It is most gratifying to note that, befitting our motto of "Physiology without borders," many of the academic activities and networking are taking place in more remote areas in the world. In addition, several national physiological societies and regional federations have broadened their outreach programs to become IUPS Initiatives. With enthusiastic and continuous support by our member societies and regional federations, I am confident that our Union will flourish in cementing collaborations in global physiology.

I applaud the addition of an eighth recommendation that "Physiologists across the globe should partner with a diverse pool of basic scientists and clinicians to spearhead research endeavors into identifying treatments and vaccines for the novel coronavirus that has caused the COVID-19 pandemic." Beyond its role as a broker of global physiology, IUPS should strive to leverage Physiology with other international unions to broker global collaboration in biomedicine toward "planetary health."

I look forward to the official launching of *Physiology: Challenges and the Way Forward* in 2022 in Beijing, China, and the implementation of the eight recommendations by the global physiological community.

Julie Y. H. Chan
President, IUPS

IUPS President Denis Noble with Julie Chan, the incumbent IUPS President, at the IUPS 38th World Congress, Rhythms of Life, Rio de Janeiro, 2017.

Incoming and Outgoing IUPS Council Members (starting top left) at the Rio de Janeiro IUPS Congress: Heikki Kainulainen, Tomasz Brzozowski, Vagner Antunes, Solusoga "Soga" Sofola, Patricia Molina, Penny Moody-Corbett, Sue Wray, Xiaomin Wang, Benedito Machado, Walter Boron, Ulrich Pohl, Rene Bindels, Yoshihiro Kubo, Penny Hanson, Denis Noble, Julie Chan, Katsuhiko Mikoshiba, Alicia Mattiazzi, Ludmila Filaretova, and Peter Hunter.

Jean François Fernel (1497 – 1558), French physician, introduced the term "physiology" to describe the study of the body's function. Image credits: Wikimedia Commons.

Executive Summary

The Board of the General Assembly (BGA) of the International Union of Physiological Sciences (IUPS) commissioned this report for assessing the global strength of physiology as a discipline. It is to be presented to the General Assembly of the 39th IUPS Congress in Beijing, China. As this report was underway, we faced the threat of the looming crisis of the pandemic over multiple waves from the novel coronavirus and its ensuing disease COVID-19. This led to the postponement of the IUPS Congress to May 2022. This report is based on responses from the IUPS member organizations to provide a picture of the field of physiology worldwide. The report also presents research articles from members of the BGA, the Executive Committee (ExCo), and the Council of IUPS that indeed reflect a kaleidoscopic view of modern day physiological sciences.

This *Report* includes five Chapters that highlight the responses we received from 25 Adhering Bodies, 9 Supporting Societies, and 2 Associate Members of the IUPS and presents to physiologists worldwide the various challenges that physiology as a discipline faces as it moves forward. Topics include academic resources, collaborative global networks, exchange of technical, and experimental models with an emphasis on translational and regenerative medicine, and the Physiome Project of the Virtual Physiological Human with applications in the healthcare needs of society. Together, member responses to these topics offer to pave a way to understand physiology as a discipline and an armamentarium against the pandemic threats arising from the severe acute respiratory syndrome (SARS-CoV-2) virus and its mutant variants. Physiologists in collaboration with immunologists, geneticists, biochemists, pharmacologists, epidemiologists, healthcare workers, and social scientists are now working at the forefront to tackle this enormous crisis to understand the basis for the viral-induced significant disruption in homeostasis.

The IUPS espouses a vision of physiology to be an excellent and well-renowned scientific discipline creating the foundations of medicine and public health that attracts the best scientists worldwide. This vision is described in the present anthology of 22 essays that reflect the vast expanse of scientific expertise of its members from the ExCo, Council, and the BGA.

COVID-19 may cause SARS resulting in tissue hypoxia and acute cardiac injury. Maintenance of adequate tissue oxygen supply in spite of rapidly changing demands is an enormous challenge for the cardiovascular system. The maintenance of vascular tone by the microvascular smooth muscle as a central effector is coordinated by a multitude of molecular mechanisms that change vascular diameter and enlist plastic remodeling of smooth muscle cells. Ulrich Pohl has addressed these issues in his article. Federico Formenti has discussed issues related to respiratory physiology for intensive care medicine that includes the development of novel oxygen sensing technology, understanding the involvement of hypoxia inducible factor in the regulation of human responses to hypoxia and in exercise physiology. Ludmila Buravkova and E.R. Andreeva further highlight the role of adipose tissue mesenchymal cells and their niche in response to hypoxia with applications to regenerative medicine in their essay.

In tandem to the observation that "when the importance of integrative systems physiology is re-emerging into the spotlight of biomedical science, the sympathetic nervous system can be viewed as the ultimate integrator of systems physiology in control of cardiovascular function,"[1] Susan Barman and Bill Yates forward a review on the sympathetic neural control of the cardiovascular system. They predict that the use of state-of-art technologies like telemetric methods for chronic recordings of sympathetic nerve activity and arterial pressure in conscious animals, coupled with optogenetics and pharmacogenetics, will help in characterizing the cardiovascular function of central neurons with known neuronal phenotypes during physiological and pathophysiological behaviors. In the operation of body functions, the control of blood pressure is one example of a multilevel integration system. Julie Chan critically examines the role of nitric oxide signaling in the integrated control of blood pressure under physiological and pathophysiological scenarios. The role of nitric oxide largely depends on its concentration and the redox environment at the site where its actions take place, and is contingent on its engagements in a physiological, pathophysiological or pathological process. The importance of salt sensitivity and the role of epithelial sodium channel showing polymorphism in establishing hypertension have been examined

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by Simiat Elias. Rene Mileva-Popova and Nina Belova discuss the impact of aging on vascular physiology. Several contemporary issues such as the escalating burden of cardiovascular disease in patients with stress, lifestyle disorders and patients with HIV on anti-retroviral therapy were addressed by Faadiel Essop.

Since alcohol abuse and HIV often coexist, Patricia Molina discusses how people on HIV anti-retroviral treatment and unhealthy alcohol consumption may suffer from gut leakage and immune activation resulting in subclinical chronic inflammation that promotes cellular energy metabolism in control (brain and pancreas), effector (liver and adipose), and target (musculoskeletal) organs and consequent increased risk for comorbidities.

The Web of Science reports a list of research topics of interest based on highly cited publications during 2015–2019, including the biology of skeletal muscle metabolism in health and disease states. Skeletal muscle metabolism changes with aging (sarcopenia) are one topic of considerable interest as discussed by Heikki Kainulainen, and by János Fodor and László Csernoch. Alberto Juan Dorta-Contreras and his associates have discussed the role of the blood-cerebrospinal fluid barrier as a molecular sieve in health and disease. Maria José Alves da Rocha comments on the likely role of statins in modulating inherent anti-oxidative and anti-inflammatory functions in septicemia. Yun Wang and her associates have addressed the issue of involvement of TRPV1 nociceptor in chronic pain and its modulation and development of analgesics targeting this receptor in their article. Yoshihiro Kubo discusses how the use of cryo-EM provides an excellent opportunity to study structural information of ion channels toward obtaining dynamic images of functioning ion channels bearing high temporal and spatial resolutions. Sung Yeon Park and Yang-Sook Chun examined the role of plant homeodomain finger protein 2 in association with several transcription factors in the epigenetic regulation of gene expression of physiological processes that include tumor suppression, adipogenesis, glucogenesis, memory formation, and osteoblast differentiation.

Rachael Augustine and Colin Brown highlight the finding of excitatory kisspeptin projection to the oxytocin system, and how the switch from prolactin inhibition to excitation of oxytocin neurons in lactation adds to the suite of regulatory mechanisms that prepare the oxytocin system for normal birth and lactation. An understanding of this process may reduce the risk of preterm labor. Endometriosis is a debilitating disease affecting many women worldwide with no known cure. Debabrata Ghosh and Jayasree Sengupta discuss how dysregulated molecular functions of physiological processes such as cell proliferation, epithelial-mesenchymal transformation, angiogenesis, apoptosis, cell survival, steroid hormone responsiveness, inflammatory and tolerogenic responses, and homeostatic stress responses known to be affected in the diseased tissue result in pain and infertility during ovarian endometriosis. The trade-off is neoplastic atypical endometriosis and its malignant transformation in high-risk women population.

Susan Wray discusses how sexual dimorphism in the availability of a key infectivity component, ACE2, necessary for COVID19 may offer a degree of protection from COVID19 in women even when confounders such as, age, infection rates, smoking, social habits, and comorbidities were considered, and suggests that heterogeneity in immune capabilities and responses may help to understand the distinct COVID19 progression in women and men and be used to guide disease prognosis and sex-specific treatments.

Peter Hunter apprises how the Physiome project of the IUPS and the launch of the journal *Physiome* encourage the publication of curated and annotated physiological models based on multiscale biophysical processes that link the organ systems of the human body to molecular mechanisms. This is accomplished through the use of models of cells, tissues and organs, as well as, models of subcellular processes such as transcription, metabolism, signaling, electrophysiology, myofilament mechanics, cell growth, and cell division which are now encoded in a markup language called CellML, developed to ensure reproducibility of these models.

The Krogh's principle named after Nobel laureate August Krogh states that "for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied."[2] This remains central to comparative biology, including neuroethology and more recently functional genomics. Global climate change has challenged researchers to study responses to changes in ecosystems and the risks of sudden and extreme weather events that are characteristic of global warming. Study of avian thermoregulation in heat and cold stresses with the use of thermal imaging and various bio-logging approaches is discussed by Liisa Peltonen and Esa Hohtola. This strategy will help to increase our understanding of

avian thermoregulatory mechanisms during such climate changes. Collaborative, interdisciplinary, and global focusing on ecosystems and impacts of their changes on avifauna may limit the collapse in avian populations due to climate change, especially in arid areas around the globe. Animal studies indeed provide essential keys to understand the biological processes involved in health and disease and allow students and researchers to determine the underlying events in a systems-wise manner. Although cell culture experiments are of great use for understanding physiology in various dimensions, they do not allow studying interactions between cells and organs and the role of the local environment for physiological functions. The use of animals for experiments is always associated with questions in animal ethics that must be attended. The Universal Declaration of the Rights of Animals by the United Nations Educational Scientific and Cultural Organization in 1978 became one important step in the recognition of the importance of living beings, aiming at an ever more ethical human behavior. Ashima Anand highlights how the Ethics Committee of the IUPS actively pursues debates, meetings, and e-learning courses for creating greater awareness of these issues within the academic community.

A cardinal feature of this report on *Physiology – Challenges and the Way Forward* prepared by the BGA of the IUPS is the following eight-point *Recommendations*. It is our hope that all societies of physiology and physiological sciences across the globe shall work closely within their own societies and interact with other groups and the IUPS to implement the *Recommendations* to advance physiology toward better health and life for all.

Recommendations

1. Societies should advocate for continued funding of basic research and collect evidence to document its state in their country
2. Networks and working groups should be created, domestically and internationally, by IUPS and member societies to facilitate the exchange of knowledge and best practice in teaching and research
3. Societies should continue the efforts of the IUPS Outreach Program to increase support among physiologists for IUPS initiatives and its furthering of the World Health Organization's *Health for All* agenda
4. Societies should implement outreach activities to raise awareness of and interest in physiology among the public and encourage the uptake of physiology and related subjects by prospective undergraduate and postgraduate students
5. Societies should develop resources to improve the teaching and learning of physiology, and to ensure graduates have a full appreciation of the complexities at all scales of physiological understanding
6. IUPS should oversee a new Global Mentorship Building Platform to facilitate Mentor/Mentee relationships among physiologists at various career stages, and in academic and clinical settings, to promote dialog and aid career development
7. Societies should explore new means to leverage funding from government and private sources, to aid the development of new initiatives designed to strengthen the discipline
8. Physiologists across the globe should collaborate with a diverse pool of basic scientists and clinicians to spearhead research endeavors into identifying treatments and vaccines for the novel coronavirus that has caused the COVID-19 pandemic. The establishment of such an alliance may prove useful to swift and efficient handling of future pandemics.

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References

1. Wallin BG, Charkoudian N. Sympathetic neural control of integrated cardiovascular function: Insights from measurement of human sympathetic nerve activity. Muscle Nerve 2019;36:595-614.

2. Krogh A. The progress of physiology. Am J Physiol 1929;90:243-51.

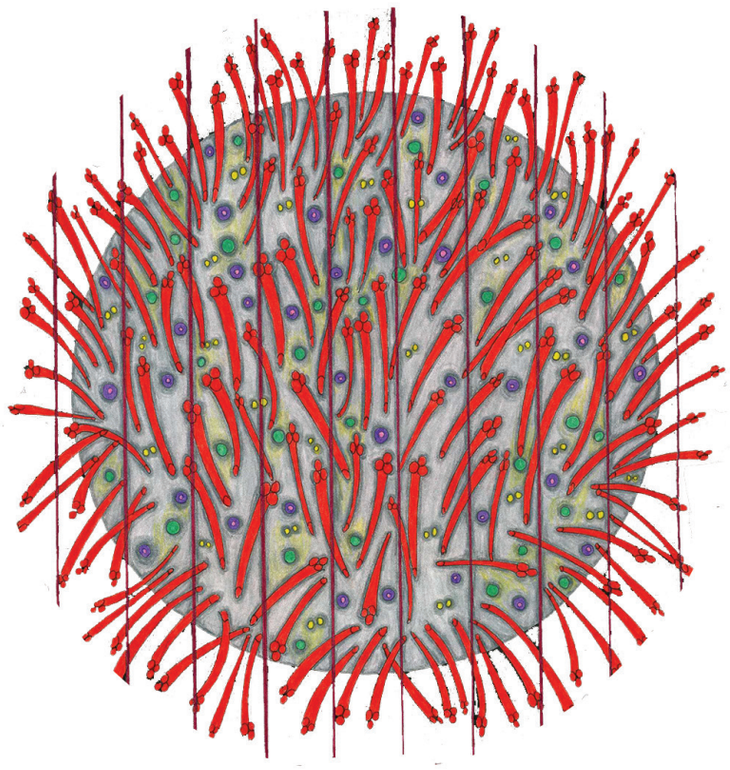
Julie Chan, President of IUPS, with participants in the IUPS Open House at Europhysiology 2018 in London, UK.

Corona virus

"when abstraction sets to killing you, you've got to get busy with it."

Albert Camus, The Plague.

And indeed we have got very busy. Through the effects of global science and global solidarity, we are putting the Covid-19 pandemic behind bars.



Artist: Anna Zeligowski, an artist and family doctor. In her pictures Anna presents a complex world of changing scenarios. Legend: Eva Jablonka, retired professor in the Cohn Institute for the History and Philosophy of Science and Ideas, Tel-Aviv University, a member of the Sagol School of Neuroscience, Tel-Aviv, and a Research Associate in the CPNSS (LSE, London University). Courtesy: Debabrata Ghosh, professor of Physiology at the All India Institute of Medical Sciences, New Delhi, India.

Claude Bernard (1813 –1878) in his laboratory (Painting by León Lhermitte 1889, Académie Nationale de Médecine, Paris). Image credits: Wikimedia Commons.

Introductory Remarks

Article V of the Constitution of the International Union of Physiological Sciences (IUPS) charges the Board of the General Assembly (BGA) with the "*responsibility to develop and present to each full meeting of the GA a written assessment of the current status of the field of physiology world-wide, emphasizing major challenges, opportunities, and problems.*" This report fulfills that responsibility and will be presented at the General Assembly of the 39th Congress of the IUPS in Beijing, China. Originally scheduled for August 2021, the meeting has been postponed until May 7 - 11, 2022, due to the 2020 pandemic.

In preparation for this report, the IUPS Manager Steven Webster emailed in April 2019 the attached IUPS BGA questionnaire to the individual identified as the contact person for IUPS member organizations. This report reflects the responses received by Spring 2020 from 36 members of the IUPS to the questionnaire that covered ten broad topics. It is hoped that this report will form the basis of a robust discussion of the current status and future goals of physiology research and teaching across the globe.

As the Chair (Jayasree Sengupta) and Vice Chair (Susan M Barman) of the BGA began preparing this report, the looming crisis of the pandemic from the novel coronavirus and its ensuing disease COVID-19 was becoming a world-wide threat. The BGA sent an additional questionnaire to IUPS member societies to ask how they have adapted to the pandemic and what they have done to assist their members in dealing with it. This questionnaire resulted in a publication in *Physiology*.[1]

The major symptoms of COVID-19 – including fever, cough, respiratory distress, muscle pain, and loss of smell and taste – signify a disruption of homeostasis. The most at-risk individuals are those with preexisting conditions such as heart disease, asthma, HIV, obesity, and diabetes that are common pathophysiological diseases across the globe. It is apparent that the elderly are very vulnerable and more likely to succumb to complications of COVID-19. Physiologists and other biomedical scientists are investigating the underlying mechanisms by which the novel coronavirus and its many variants attack the immune system such as the destruction of T cells that function to protect the body from harmful invaders and why a subset of population remains unaffected or minimally affected despite confirmed infection.

Physiological Societies across the globe – including the American Physiological Society and The Physiological Society – have provided their members with resources to help guide their work to discover the pathophysiological basis for COVID-19 and why the physiological changes associated with aging are so significant, the development of more effective testing for the presence of the virus and its antibodies, and the discovery of effective treatments and vaccines. This is a time for physiologists across the globe to unite in their efforts to prevent more victims of this devastating pandemic and to bring us back to the *Wisdom of the Body* as described by the eminent American physiologist Walter B. Cannon in his book with that title first published in 1932.

[1]Barman SM, Csernoch L, and Sengupta J. Physiological societies across the globe unite in an effort to handle the COVID-19 pandemic. Physiology 2021;36:62-70.

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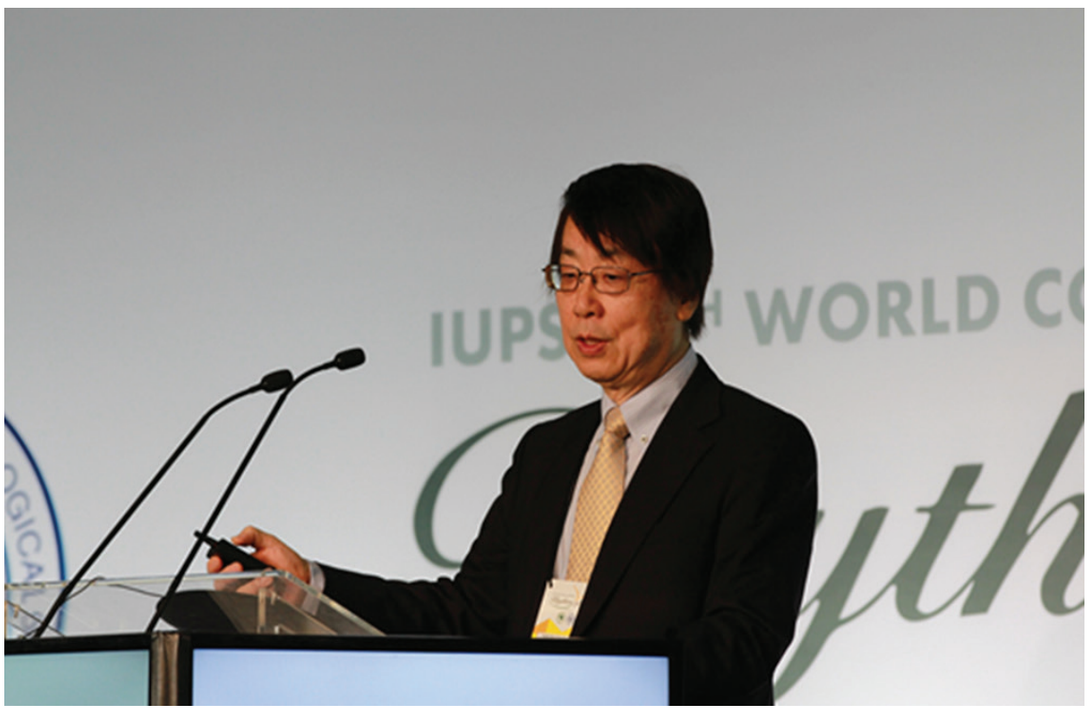

Plenary lecture delivered by Yasushi Miyashita (Japan) on "Neural dynamics of cognitive memory in the primate-where global network meets local circuits" at the 38th IUPS Congress.

Plenary lecture delivered by Amira Klip (Canada) on "Immune cells co-opting metabolism to cause insulin resistance" at the 38th IUPS Congress.

Introduction

This chapter reflects the responses to four topics covered by the questionnaire that was distributed to IUPS member organizations across the world. These topics included questions designed to gather information on the profile of physiological societies that are members of the IUPS, career opportunities for physiologists represented by these societies, engagement of member organizations with other relevant groups, the value of IUPS membership to physiologists across the globe, and an open-ended request for issues that should be brought to the attention of the leadership of the IUPS.

Profile of Responding IUPS Member Physiological Societies

The IUPS is comprised 45 Adhering Bodies, 17 Supporting Societies, 5 Regional Members, 13 Associate Members, and 2 Affiliated Societies. By Spring 2020, we had received responses to the questionnaire from 25 Adhering Bodies, 9 Supporting Societies, and 2 Associate Members representing physiologists worldwide [Table 1]. The responding physiological organizations vary in size from nearly 8000 members (American Physiological Society) to only 25 members (Physiological Society of Nepal) with an average of 831 members that includes regular and undergraduate and/or graduate student members in most cases (29 of 36 responding organizations). Four societies (Brazilian Physiological Society, French Society of Physiology, Mexican Society of Physiological Sciences, and The Physiological Society) have more student members than regular members. The geographical distribution of the responding physiological societies is shown in page 36.

Career Opportunities

The members of the IUPS were asked about career options for the physiologists in their geographical region. They were asked to choose from ten possibilities: Research in Academia, Teaching, Research in Industry, Academic Administration, Industry Administration, Science Advocacy, Science Consultant, Science Writer, Government, and Medical Devices [Table 2]. Ten of the responding societies indicated that all the options were available for physiologists in their region; this included societies from Africa, The Americas, Asia, and Europe/European Union. The average number of career options for physiologists represented by the 36 responding organizations was six. The most limiting prospects for career choices were in Nepal where physiologists have only one option; that is to pursue a career as a teacher. The remaining 35 responders indicated that research in academia was a career choice for physiologists in their region. The next likeliest career choice was teaching (33 responders) which was followed by academic administration (25 responders), research in industry (24 responders), and medical devices (21 responders). The remaining five career choices are career options for physiologists represented by fewer than 20 societies: Industry administration $n = 15$), science advocacy ($n = 14$), science consultation ($n = 19$), science writer ($n = 18$), and government ($n = 19$).

Engagement of Other Relevant Bodies in the Pursuit of Research Skills, Learning, and Careers in the Physiological Sciences

IUPS members were asked to describe how other groups in their region contribute to the pursuit of research, learning, and career strategies in physiological and life sciences. Most IUPS member physiological societies acknowledge that they do not work in isolation to advance research, teaching, and careers in physiology. Table 3 lists the responses from 29 members of the IUPS. Eight of the responders reported the important roles of universities in promoting the development of skills and careers in the physiological sciences. Six physiological societies remarked that its members interacted with organizations involved in the neurosciences. Five member organizations noted the importance of government resources to raise awareness of physiological sciences.

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Table 1: Characteristics of responding IUPS member organizations.

Geographical location	IUPS membership category	Membership numbers Regular/Graduate/ Undergraduate/Affiliate
Africa		
Physiological Society of Nigeria	Adhering Body	300/120/-/-
Physiological Society of Southern Africa	Adhering Body	82/56/-/44
The Americas		
American Physiological Society	Adhering Body	6029/1358/252/-
Argentinean Society of Physiology	Adhering Body	200/100/-/95
Brazilian Physiological Society	Adhering Body	350/650/300/150
Canadian Physiological Society	Supporting Society	127/152/-/52
Chilean Society of Physiological Sciences	Adhering Body	150/-/1/10
Cuban Society of Physiological Sciences	Adhering Body	90/30/-/-
Mexican Society of Physiological Sciences	Adhering Body	60/90/-/25
Asia		
Association of Physiologists and Pharmacologists of India	Supporting Society	3017/-/-/-
Bangladesh Society of Physiologists	Associate Member	281/154/-/-
Chinese Association for Physiological Sciences	Adhering Body	720/410/-/-
Chinese Physiological Society in Taipei	Adhering Body	350/300/-/-
Korean Physiological Society	Adhering Body	152/149/-/-
Pakistan Physiological Society	Adhering Body	249/-/-/-
Physiological Society of India	Supporting Society	800/50/-/-
Physiological Society of Japan	Supporting Society	2316/405/-/97
Physiological Society of Nepal	Associate Member	25/-/-/-
Russian Academy of Sciences, Russian Physiological Society	Adhering Body	720/280/-/-
Europe/European Union		
Bulgarian Society for Physiological Sciences	Adhering Body	95/10/-/15
Czech Physiological Society	Adhering Body	187/-/-/-
Finnish Physiological Society	Adhering Body	89/-/2/30
French Society of Physiology	Supporting Society	200/300/50/100
German Physiological Society	Adhering Body	583/24/32/0
Hungarian Physiological Society	Adhering Body	468/53/-/25
Life Science Switzerland (LS2)-Physiology	Supporting Society	119/78/3/-
Romanian Society of Physiology	Adhering Body	241/-/-/-
Russian Academy of Sciences, Russian Physiological Society	Adhering Body	720/280/-/-
Slovak Physiological Society	Adhering Body	136/28/-/-
Slovenian Physiological Society	Adhering Body	50/30/30/-
Spanish Society of Physiological Sciences	Adhering Body	400/100/100
The Physiological Society	Adhering Body	1691/947/1170/60
Turkish Association of Physiological Sciences	Adhering Body	270/130/-/-

(*Contd...*)

Table 1: (*Continued*).

Geographical location	IUPS membership category	Membership numbers Regular/Graduate/ Undergraduate/Affiliate
Mideast		
Iranian Society of Physiology and Pharmacology	Adhering Body	129/122/-/-
Israel Society of Physiology and Pharmacology	Supporting Society	60/-/-/-
Oceania		
Australian Physiological Society	Supporting Society	250/157/-/60
Physiological Society of New Zealand	Supporting Society	52/27/-/-

At least 11 IUPS member organizations report joint ventures with other scientific bodies in the pursuit of research, teaching, and career development. The American Physiological Society noted the importance of engagement with "*other learned societies whose members pursue physiological research but do not self-identify as physiologists.*" As a major member under the umbrella of Federation of American Societies of Experimental Biology, they collaborate with members of other societies "*in co-sponsorship ventures such as workshops and symposia at EB and other meetings.*" Likewise, the Chinese Physiological Society in Taipei joined the Federation of the Asian and Oceanian Physiological Societies "*to promote physiological education, learning, and research.*" The Israel Society of Physiology and Pharmacology indicated that there are "*more than 30 societies related to physiology that gather for a big meeting*" every 3 years. The Physiological Society interacts with the Royal Society of Biology to "*offer professional registration for technicians and scientists in the UK*" and with the British Pharmacological Society "*to develop a curriculum to support in vivo skills development.*"

Value of IUPS Membership

The responding physiological organizations were asked to reflect on what they conceived as the major value of being a member of the IUPS. Table 4 shows their responses. The most common reply shared by at least 25 responding societies was that membership in the IUPS provides a venue for networking and interacting with physiologists across the globe to exchange research and teaching knowledge. Related to this, at least 11 societies noted that meetings, including the IUPS Congress, were valued because they are a way to share research and teaching skills and to learn new technologies along with other international physiologists.

At least nine of the responding organizations commented that membership in the IUPS promotes the mutual understanding of the trends and challenges facing physiological researchers and educators across the globe. At least six of the member societies noted that IUPS membership boosts the recognition of their own members at an international level. For example, the French Physiological Society wrote that it "*Gives us the credibility that we belong to an International Organization and we can seek its help.*" The Cuban Society of Physiological Sciences wrote that IUPS membership is a way "*to contribute to the integration of Cuba to the rest of the world.*" The Bangladesh Society of Physiologists further commented that membership enables them to identify their weakness and deficiencies and drive them to find a way to overcome these handicaps.

Recommendations to the IUPS to Promote Physiology Globally

The IUPS member organizations were asked to reflect on what they thought the IUPS could do to strengthen physiology worldwide. Table 5 lists the recommendations offered by 31 responding Societies. The American Physiological Society articulated a concept echoed by other Societies by noting that "*The IUPS can play a particularly valuable role supporting physiology in developing countries, providing expertise, coordinating knowledge exchange, and perhaps providing grants and awards to researchers and educators.*" The most common suggestions were related to promoting interactions

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Table 2: Career opportunities of physiologists across the globe.

Member Organization	Researcher in Academia	Teacher	Researcher in Industry	Academic Administration	Industry Administration	Science Advocacy	Science Consultant	Science Writer	Government	Medical Devices
American Physiological Society	*	*	*	*	*	*	*	*	*	*
Argentinean Society of Physiology	*	*		*						
Association of Physiologists and Pharmacologists of India	*	*								
Australian Physiological Society	*	*							*	
Bangladesh Society of Physiologists	*	*		*						
Brazilian Physiological Society	*	*							*	
Bulgarian Society for Physiological Sciences	*	*	*	*	*					*
Canadian Physiological Society	*	*	*	*	*	*	*	*	*	*
Chilean Society of Physiological Sciences	*	*								
Chinese Association for Physiological Sciences	*	*	*	*		*	*	*		*
Chinese Physiological Society in Taipei	*	*	*	*	*	*	*	*	*	*

(*Contd...*)

Table 2: (*Continued*).

Member Organization	Researcher in Academia	Teacher	Researcher in Industry	Academic Administration	Industry Administration	Science Advocacy	Science Consultant	Science Writer	Government	Medical Devices
Cuban Society of Physiological Sciences	*	*	*	*		*	*		*	
Czech Physiological Society	*	*	*	*		*	*	*	*	*
Finnish Physiological Society	*	*	*	*	*	*	*	*	*	*
French Society of Physiology	*		*							*
German Physiological Society	*		*	*	*					
Hungarian Physiological Society	*	*					*			
Iranian Society of Physiology and Pharmacology	*	*	*	*			*	*		*
Israel Society of Physiology and Pharmacology	*	*	*	*	*		*	*		*
Korean Physiological Society	*	*	*	*	*	*	*	*	*	*
Life Science Switzerland (LS2)-Physiology	*	*	*	*	*	*	*	*	*	*
Mexican Society of Physiological Sciences	*								*	

(*Contd...*)

Table 2: (*Continued*).

Member Organization	Researcher in Academia	Teacher	Researcher in Industry	Academic Administration	Industry Administration	Science Advocacy	Science Consultant	Science Writer	Government	Medical Devices
Pakistan Physiological Society	*	*		*				*		
Physiological Society of India	*	*	*	*	*	*	*	*	*	*
Physiological Society of Japan	*	*	*	*	*					
Physiological Society of Nepal		*								
Physiological Society of New Zealand	*	*	*	*				*		*
Physiological Society of Nigeria	*	*	*	*		*	*	*	*	*
Physiological Society of Southern Africa	*	*	*	*	*	*	*	*	*	*
Romanian Society of Physiology	*	*								
Russian Academy of Sciences, Russian Physiological Society	*	*	*	*	*		*	*	*	*
Slovak Physiological Society	*	*	*							*
Slovenian Physiological Society	*	*	*						*	

(*Contd...*)

Table 2: (*Continued*).

Member Organization	Researcher in Academia	Teacher	Researcher in Industry	Academic Administration	Industry Administration	Science Advocacy	Science Consultant	Science Writer	Government	Medical Devices
Spanish Society of Physiological Sciences	*	*	*	*	*	*	*	*	*	*
The Physiological Society	*	*	*	*	*	*	*	*	*	*
Turkish Association of Physiological Sciences	*	*		*			*		*	*

between physiologists and physiological societies across the globe (noted by 11 Societies) and in providing grants or funds to support such interactions (requested by ten responders). Seven Societies remarked that it was important to include developing countries in such activities.

A recommendation of the Physiological Society of India resonated with that of ten responders. They requested that the IUPS help with "*organizing conferences/seminars/workshops/symposia on different aspects of physiological sciences to popularize it among the young generation.*" The suggested formats for these programs included skill development workshops, physiological education and training courses, and online learning modules.

Three of the responding societies highlighted the need to educate the public about the importance of physiology. The Hungarian Physiological Society requested that the IUPS "*clarify for the public that understanding the healthy operation of living organisms is essential for understanding the nature and behavior of any disease.*" They suggested that one way to accomplish this would be to provide "*detailed publications of the history of physiology and discoveries that changed the entire way of thinking on health and disease.*" This view was reinforced by the Czech Physiological Society who wrote that the IUPS should "*provide the public accessible educational materials on different level which includes the word 'physiology' to improve awareness about physiology.*" Likewise, the Slovak Physiological Society requested that the IUPS "*provide public propagation of 'interesting' physiological discoveries by all possible media means.*"

At least eight societies promoted strengthened communication about educational and research resources available to all physiologists. For example, the Iranian Society of Physiology and Pharmacology suggested that the IUPS could use their website "*to offer enriched resources for physiological techniques, introducing new concepts, online advanced courses, and new directions for physiological researches.*" The Physiological Society of New Zealand suggested that the IUPS could "*profile what physiologists do using social media to connect with next generation of researchers.*"

Five of the responding societies commented on the need for the IUPS to be an advocate for funding and conducting translational research including that involving the use of animals. Other recommendations were for the IUPS to "*create sub-groups of each commission to represent all the sub-divisions of physiology*" (French Society of Physiology), "*put more effort for physiology to be an applied science not a*

Table 3: Regional groups engaged in research, learning, and careers in physiological and life sciences.

Member Organization	Regional organization engagement in advancing physiological and life sciences
American Physiological Society	• There are many other learned societies whose members pursue physiological research but do not self-identify as physiologists. We collaborate with them in co-sponsorship ventures such as workshops and symposia at EB and other meetings
Argentinean Society of Physiology	• There are several organizations related to life sciences, like the Biophysics Society or the Society of Biochemistry that are involved in the development of research skills, but unsure whether they pursue learning and career strategies
Association of Physiologists and Pharmacologists of India	• India Society for Sleep Research and Indian Academy of Neurosciences have been conducting skill development workshops for technologists and researchers in respective areas with faculty participation from India and abroad
Bangladesh Society of Physiologists	• Several government-run institutes under the ministry of health, such as center of medical education, Bangladesh medical research council, public medical university, promote research and academic skill in physiology, and other health sciences in Bangladesh
Bulgarian Society for Physiological Sciences	• Through opening of academic and universities research and/or teacher's positions, go beyond the surface, use the networks
Canadian Physiological Society	• There are many institutions affiliated with CPS that assist in the pursuance of physiological sciences, including Canadian universities, hospitals, and societies such as Canadian Association of Neuroscience
Chilean Society of Physiological Sciences	• In Chile Universities oversee this concept. Industry is scarcely involved
Chinese Association for Physiological Sciences	• Conducting summer training courses on scientific paper and fund writing, classroom teaching, and experimental teaching of physiology
Chinese Physiological Society in Taipei	• CPS joined Federation of the Asian and Oceanian Physiological Societies to promote physiological education, learning, and research • We cooperate with biotech companies and government to raise the awareness of incorporating physiological parameters in developing new medical devices and new drugs
Cuban Society of Physiological Sciences	• APS should continue to support the regional advances • The recent structured Sociedad Latinoamericana de Ciencias Fisiolgogicas can contribute to this purpose
Czech Physiological Society	• CPS cooperates with multiple other societies in the framework of Czech Medical Association of Jan Evangelista Purkyne, including Czech Neuroscience Society and Czech Psychiatric Society
Finnish Physiological Society	• It is common that university campuses are "academic centers" comprised of learning and research programs, research institutes, innovation services, biobanks, and bio(medical) companies • Within the medical sciences, university hospitals and districts belong tightly to these networks; few examples, Turku Health Campus, The Academic Medical Center Helsinki, Medical Research Center Oulu, OuluHealth, Helsinki One Health (translational approach, problems concerning animal and human populations worldwide), Tampere University Centre of Body-on-Chip Research

(*Contd...*)

Table 3: (*Continued*).

Member Organization	Regional organization engagement in advancing physiological and life sciences
Hungarian Physiological Society	• Hungarian Physiological Society has an almost 90-year history (founded in 1931) based on collaborations with a number of sister societies • We have organized joint meetings and had FEPS and IUPS conferences in Budapest and satellite meetings in various Hungarian cities (pecs, Szeged, Debrecen, Tihany) with strong international participation • Our students and scientists are embedded in various International Research and Education programs
Iranian Society of Physiology and Pharmacology	• Federation of Iran Bioscience Society and Iranian Scientific Association of Clinical Laboratory are involved in introducing and developing the Physiological Sciences and have collaboration with ISPP in providing job opportunities for Physiology and Pharmacology alumni
Israel Society of Physiology and Pharmacology	• We have more than 30 societies related to physiology that gather for a big meeting every 3 years; all societies are engaged in the above activities mainly through actions of individual members, similarly to our own Society
Korean Physiological Society	• Our society is a member of Korean Academy of Medical Sciences • *The Korean Journal of Physiology and Pharmacology* has been co-published • Previously, *The Korean Journal of Physiology* has been published since 1965
Life Science Switzerland (LS2)-Physiology	• Multidisciplinary PhD programs (i.e., PhD in Biomedicine which combines the [former] integrative and molecular medicine program and the translational biology program) are developed in some universities, for example, at UZH/ETHZ. This kind of program may be developed further
Mexican Society of Physiological Sciences	• We have only a few non-governmental associations in this issue
Pakistan Physiological Society	• There exist some other Professional Societies and Governmental Authorities involved in pursuance of learning and research, but unfortunately most of them at the Society level have meager resources • The governmental authorities like Higher Education Commission, Pakistan Science Foundation, Pakistan Medical Research Council and National Institute of Health are functioning independently and have little coordination among themselves, resulting in wastage of resources at times
Physiological Society of India	• Universities: Calcutta, Vidyasagar, Tripura, Burdwan, North Bengal, WB State, etc. Research Institute: DIPAS, DRDO, Bose Institute, IICB, CNCRI, IISER, NICED, NISER, NCBS, TIFR, NBRC, NIBMG, IISC, etc. • Medical Colleges: PG-Kolkata, CNRI- Kolkata, AIIMS-New Delhi, PGI-Chandigarh, NIMHANS- Bangalore, King George- Lucknow, etc.
Physiological Society of Japan	• There are other academic societies in the basic life science research field, such as societies for Pharmacology, Anatomy, Biochemistry, Molecular Biology, Biophysics, and Neuroscience. All share the aim to understand the mechanism of function of life • PSJ organizes interdisciplinary symposiums with these societies at annual meetings

(*Contd...*)

Table 3: (*Continued*).

Member Organization	Regional organization engagement in advancing physiological and life sciences
Physiological Society of Nepal	• PSN is the only organization directly related to physiological and life sciences in Nepal, other closest being clinicians' organizations/societies • Various medical colleges provide some platform and environment to conduct research works in different fields, mostly descriptive types and infrequently interventional/experimental studies
Physiological Society of New Zealand	• Through government funding Centers of Research Excellence there has been investment into heart and brain research
Physiological Society of Southern Africa	• International Brain Research Organization and the South African Heart Association run workshops and demonstrations to undergraduate students • There are also health sciences education workshops that are organized locally by the South African Association of Health Educationalists
Slovak Physiological Society	• Universities and Academy of Sciences through pregraduate and postgraduate study programs, postdoc, and researcher positions • Minor role in this is played by Slovak Society for Pathological and Clinical Physiology and Slovak Society for Physiology and Pathology of Breathing
Slovenian Physiological Society	• Slovenian Biochemical Society • Slovenian Biophysical Society • SiNAPSA
Spanish Society of Physiological Sciences	• There is a meeting of scientific societies called COSCE to which the Spanish Society of Physiological Sciences belongs
The Physiological Society	• We work with the Royal Society of Biology who offer professional registration for technicians and scientists in the UK • We work with the British Pharmacological Society to develop a curriculum to support in vivo skills development
Turkish Association of Physiological Sciences	• Turkish Respiratory Society has a Respiratory Physiology branch in pursuit of learning and skills in the subject

textbook science" (Korean Physiological Society), "*encourage physiology growth in regions where it is not active as societies*" (Physiological Society of Southern Africa), and "*provide a platform for dialogue at bilateral and multilateral levels by continuing to facilitate meetings and collaboration between countries*" (The Physiological Society).

The Bangladesh Society of Physiologists noted that the "*IUPS is already playing vital role in the progression of global physiology.*" After remarking that "*The leadership in the Physiome research field has been visible and will continue to be beneficial,*" the Physiological Society of Japan remarked that a search "*for new interdisciplinary research fields will be important.*" Examples of new fields included the interaction of life and the environment and life in extreme environments. Both the American Physiological Society and the Chinese Association of Physiological Sciences remarked that they welcome the opportunity to work together with the IUPS to promote its initiatives.

Additional Suggestions

The physiological organizations comprising the IUPS were also given an opportunity to identify other issues they would like to bring to the attention of the IUPS. Comments were received from 11 physiological societies (see Table 6). Some of these suggestions reiterated the recommendations designed to

Table 4: Value of being a member of the IUPS.

Member Organization	Value of IUPS Membership
American Physiological Society	• To enhance global outreach for the discipline
Argentinean Society of Physiology	• To be able to share with physiologists all around the world their experiences as researchers and teachers
Association of Physiologists and Pharmacologists of India	• Scientific networking at a global level • Gaining awareness of the trends and challenges in physiological sciences
Australian Physiological Society	• To be part of the peak body that fosters the study of physiology worldwide • To provide opportunities to our members internationally for the dissemination and production of knowledge in the field of physiology
Bangladesh Society of Physiologists	• Exposure to global trends, challenges, and update information about the activities in the world of Physiology • Opportunity to meet and communicate with the members of physiological societies from all over the world • Opportunity to share academic and research knowledge and experience • Enable us to identify our weakness and deficiency and drive us to find a way out
Brazilian Physiological Society	• Opportunity to participate in the scientific meeting organization
Bulgarian Society for Physiological Sciences	• Participation in meetings and conferences in physiology • Contacts and knowledge exchange
Canadian Physiological Society	• To promote the CPS and Canadian physiological research on an international level • To have a greater opportunity for international collaboration • To promote Canadian research community and facilities
Chilean Society of Physiological Sciences	• The possibility of linking with other societies
Chinese Association for Physiological Sciences	• CAPS will give the strongest support to IUPS in promoting the discipline of physiology worldwide
Chinese Physiological Society in Taipei	• Increased international visibility of a regional physiological society • Scientific information exchange, interactions, and collaborations • Understanding the global progress of physiological sciences
Cuban Society of Physiological Sciences	• To expand our knowledge about the physiology in other parts of the world • To contribute to the integration of Cuba to the rest of the world
Czech Physiological Society	• Access to resources of the organization • Contact with other members • Exchange of ideas and methods
Finnish Physiological Society	• Regional and global networking of physiologists
French Society of Physiology	• Gives us the credibility that we belong to an International Organization and we can seek its help, if required
German Physiological Society	• As the worldwide umbrella organization of physiology, the IUPS offers the only platform to present the German physiological science in direct comparison.
Hungarian Physiological Society	• Being updated with relevant information
Iranian Society of Physiology and Pharmacology	• Improving the international activities • Collaboration with IUPS to improve the education and research in Physiology

(*Contd...*)

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Table 4: (*Continued*).

Member Organization	Value of IUPS Membership
Israel Society of Physiology and Pharmacology	· Mainly participation in IUPS meetings
Korean Physiological Society	· International communication in physiology field
Life Science Switzerland (LS2)-Physiology	· Scientific interaction
Mexican Society of Physiological Sciences	· Opportunity to carry out research collaborations and knowledge in the area
Pakistan Physiological Society	· International exposure and introduction · Participation in international conferences · Exposure to advances in the field of Physiological Sciences · Exchange of experiences · Learning new techniques in Physiology and Physiology Teaching · Access to IUPS publications · Mutual understanding of the problems
Physiological Society of India	· To be aware of the advancement of physiological sciences at international level and the activities of different countries · To organize the Annual Conferences of the society in collaboration with IUPS · To participate in the conferences organized by IUPS
Physiological Society of Japan	· To know the situation of other physiological societies · To interact with the other societies · To work together for the promotion of research and education of physiological sciences
Physiological Society of Nepal	· Be aware of the activities happening in physiological sciences around the world · For scientific gatherings · To facilitate our members to participate in such events as far as possible
Physiological Society of New Zealand	· Being part of a global network of physiological societies · Being able to enhance research in New Zealand
Physiological Society of Nigeria	· To foster collaborations between physiologists of different countries · To promote international congresses which bring scientists from different countries together
Physiological Society of Southern Africa	· Networks · Funding · Capacity building
Romanian Society of Physiology	· Participation in IUPS congresses and IUPS-sponsored regional and international workshops and conferences on physiology and physiology education
Russian Academy of Sciences, Russian Physiological Society	· Unfortunately, such advantages as receiving information prior to publication are shrinking · No other advantages
Slovak Physiological Society	· Contacts · Information exchange · Possibility to improve understanding of what is physiology by public

(*Contd...*)

Table 4: (*Continued*).

Member Organization	Value of IUPS Membership
Slovenian Physiological Society	• To be in contact and share knowledge with physiology community in physiological societies outside Europe
Spanish Society of Physiological Sciences	• Bring together people with experience in all branches of science, especially in Biology • Motivated with enthusiasm and desire to transmit their experiences • Access to material and teaching advice
The Physiological Society	• Working in collaboration with partner societies across the world on our common interests
Turkish Association of Physiological Sciences	• To improve teaching and learning of physiology accomplished mostly by the Medical Schools • Opportunities for workshops and other events during congresses

Table 5: Recommendations for how the IUPS could strengthen physiology globally.

Member Organization	Suggestions for the IUPS
American Physiological Society	• Become more self-sustaining, relying less on large member societies like APS and TPS to fund its activities • Play a particularly valuable role supporting physiology in developing countries, providing expertise, coordinating knowledge exchange, and perhaps providing grants and awards to researchers and educators • Raise funds to support these last two activities • The APS welcomes the opportunity to work together on these important issues
Argentinean Society of Physiology	• Strengthen the communication with the different physiological societies
Association of Physiologists and Pharmacologists of India	• Collaborate with supporting societies to conduct skill development workshops and symposia in different continents/regions on specific areas being identified by the supporting societies as per their national health policies with participation of expert resource faculty from across the globe
Bangladesh Society of Physiologists	• IUPS is already playing vital role in progression of global physiology
Brazilian Physiological Society	• Support Brazilian physiologists to be abroad during meetings to experience research activities in countries that are not under the stress that Brazilians are experiencing because of their economy and political situation
Bulgarian Society for Physiological Sciences	• Acquire funds to help physiological scientists in developing countries and those in low/middle economy levels
Canadian Physiological Society	• Offer smaller societies, such as CPS, further opportunities to collaborate with other researchers to help strengthen profiles and representation on a global level
Chilean Society of Physiological Sciences	• Support local initiatives • Support internationalization of emerging countries

(*Contd...*)

Table 5: (*Continued*).

Member Organization	Suggestions for the IUPS
Chinese Association for Physiological Sciences	• Conduct physiological education and training courses around the world
Chinese Physiological Society in Taipei	• Provide the opportunity for physiologists to strengthen the linkage with Industry • Emphasize the importance of animal research in physiological sciences
Cuban Society of Physiological Sciences	• Help Cuba and other countries to integrate with the rest of the world
Czech Physiological Society	• Try to improve efficacy of translation of knowledge to clinics • Provide the public accessible educational materials on different level which includes the word "Physiology" to improve awareness about Physiology
Finnish Physiological Society	• Inform members on available funding for international collaborative projects in teaching and research in the field of physiology • Advocate for basic research funding • Support regional IUPS Initiatives in teaching and research
French Society of Physiology	• Create sub-groups of each commission to represent all the sub-divisions of physiology
German Physiological Society	• Collect as much money as possible to provide travel grants to IUPS meeting
Hungarian Physiological Society	• Clarify for the public that understanding the healthy operation of living organisms is essential for understanding the nature and behavior of any disease • Raise visibility that physiology research requires funding although its translational value is less obvious as compared to disease-oriented research • Education programs for the media and detailed publications of the history of physiology and discoveries that changed the entire way of thinking on health and disease
Iranian Society of Physiology and Pharmacology	• Provide situations for more scientific collaborations between physiologists all over the world • Distribute its scientific activities to all countries including well-developed and developing countries • Use IUPS website to offer enriched resources for physiological techniques, introducing new concepts, online advanced courses, and new directions for physiological researches
Korean Physiological Society	• Put more effort for physiology to be an applied science not a textbook science
Life Science Switzerland (LS2)-Physiology	• Establish a collaborative program on education (on-line learning program) and research on physiology.
Mexican Society of Physiological Sciences	• Hold regional congresses • Support exchange programs for teachers and students • Sponsorship of teaching and dissemination programs of physiological sciences as the Society for Neuroscience does

(*Contd...*)

Table 5: (*Continued*).

Member Organization	Suggestions for the IUPS
Pakistan Physiological Society	• Spread the knowledge and skills achieved by the members globally • The publications of IUPS must be freely accessible to all members • Help the member Societies in holding training workshops and symposia in their field for promotion of physiology and life sciences globally • Come forward and patronize regional scientific publications in the field of physiology and life sciences • Support ISI indexation fees for PubMed by trying to waiver such fees or helping through matching grants
Physiological Society of India	• Support for organizing conferences/seminars/workshops/symposia on different aspects of physiological sciences to popularize it among the young generation in different parts of India; if possible, with partial financial assistance under the banner of IUPS
Physiological Society of Japan	• At the global level, world-wide activity of the physiology education could be very important and effective as ever • The leadership in the Physiome research field has been visible and will continue to be beneficial • A searching activity for new interdisciplinary research fields will be important; interaction of life and environment, life in extreme environment are examples
Physiological Society of Nepal	• Play the linking role between societies in developed and other countries to take physiology research to the developing and low- mid-income countries
Physiological Society of New Zealand	• Profile what physiologists do using social media to connect with next generation of researchers
Physiological Society of Nigeria	• Strengthen and encourage more collaborations between physiologists in the first world and the developing countries • Provide information on a simple approach for carrying out physiological experiments in resource-challenged environment
Physiological Society of Southern Africa	• Networks, funding, equipment, telecommunications, and online resources • Encourage physiology growth in regions where it is not active as societies
Russian Academy of Sciences, Russian Physiological Society	• Support joint international grants with obligatory interchange of PI and young scientists between countries
Slovak Physiological Society	• Provide public propagation of "interesting" physiological discoveries by all possible media means.
The Physiological Society	• Provide a platform for dialogue at bilateral and multilateral levels by continuing to facilitate meetings and collaboration between countries
Turkish Association of Physiological Sciences	• Become more active by arranging educational and teaching activities in different locations with a collaboration of local physiological societies

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Table 6: Additional issues to consider.

Member Organization	Suggestions for the IUPS
American Physiological Society	· Continue to promote the discipline but try to encourage a broader range of scientific expertise on its governing body · Encourage the development of interdisciplinary meetings that bring together the best basic, translational and clinical scientists, irrespective of their self-identification as physiologists
Association of Physiologists and Pharmacologists of India	· Identify target area for new job opportunities for physiologists in academia and industry · Bring proactive changes in physiology education to meet the requirements of the new job profiles · Skill development and manpower training in the field of systems physiology
Bangladesh Society of Physiologists	· IUPS can give more attention to the promotion of physiology education and research especially in under privileged developing countries · Sending resource persons to train and educate or help providing scholarship or fellowship for training on technical skill in developed countries
Chinese Association for Physiological Sciences	· Helping the development of CAPS · Expanding the impact of CAPS in IUPS · Strengthening the collaboration with multiple physiology societies around the world.
Chinese Physiological Society in Taipei	· Raise the awareness of basic research in women's health-related diseases · Hold regional and/or local workshops regarding employing innovative techniques in physiological research
Mexican Society of Physiological Sciences	· Joint participation in the activities of the company
Pakistan Physiological Society	· In some countries (including ours), the basic medical sciences are a neglected entity, especially today when science is rapidly changing · IUPS can launch global projection of the Basic Life Sciences as foundation to all medical and life sciences · The vanishing individual and independent identity of the subjects must be restored for their survival and existence as a subject · The medical publications from developing countries must be helped to gain their due place in the sea of global publications

(*Contd...*)

Table 6: (*Continued*).

Member Organization	Suggestions for the IUPS
Physiological Society of India	· Basic physiologists (MSc, PhD under the Faculty of Science) are not allowed to teach in Medical Colleges as per norms of Medical Council of India · Physiology as a basic science subject is taught in many colleges and universities in India for more than 100 years, but it is yet to be included in the list of University Grants Commission (UGC) approved subjects, unlike its allied life science subjects · Now in West Bengal, besides University of Calcutta, Physiology as basic science is taught in many other Universities and their affiliated colleges, including Burdwan University, Vidyasagar University (Midnapore), West Bengal State University in Barasat, North Bengal University (only at UG level) in Jalpaiguri, and Kalyani University. · Total no. of colleges where physiology is taught at UG level is now about 100 · Besides, Physiology as basic science other than West Bengal is taught in Tripura University (a Central University) and its most affiliated colleges about 20 in number · Besides West Bengal and Tripura, no other states/Union territories in India where Physiology is taught under the faculty of basic science · The students who completed MSc and PhD in Physiology under the faculty of basic sciences are not getting proper job opportunities as the ratio of pass out students from the universities is much less than uptake in colleges, universities, and research institutes · Besides the medical colleges of India are now not recruiting MSc/PhD students from basic physiology. Earlier there was provision of Indian Medical Council of 33% reservation from basic physiology; however this provision is no more though from The Physiological Society of India (PSI) we made several representations from 2016 to 2019 and two court cases in High Court filed against AIIMS advertisements for AIIMS Kalyani and AIIMS at Nagpur & Mangalaguri. All the letters written on behalf of PSI to the then MCI, Nitiayog's PMO, Ministry of Health, Govt. of India, Justice Lodha who has been looking after the matter, Higher Education Minister, Govt. of India, etc.
Physiological Society of Japan	· From the point of view of healthy and peaceful human life, the activity of IUPS on the global environment including temperature elevation could be important
Physiological Society of Nepal	· Many of our members hope that they get exposures to the know-how of physiology researchers by being associated with organizations like the IUPS
Russian Academy of Sciences, Russian Physiological Society	· Find a way to support its initiatives

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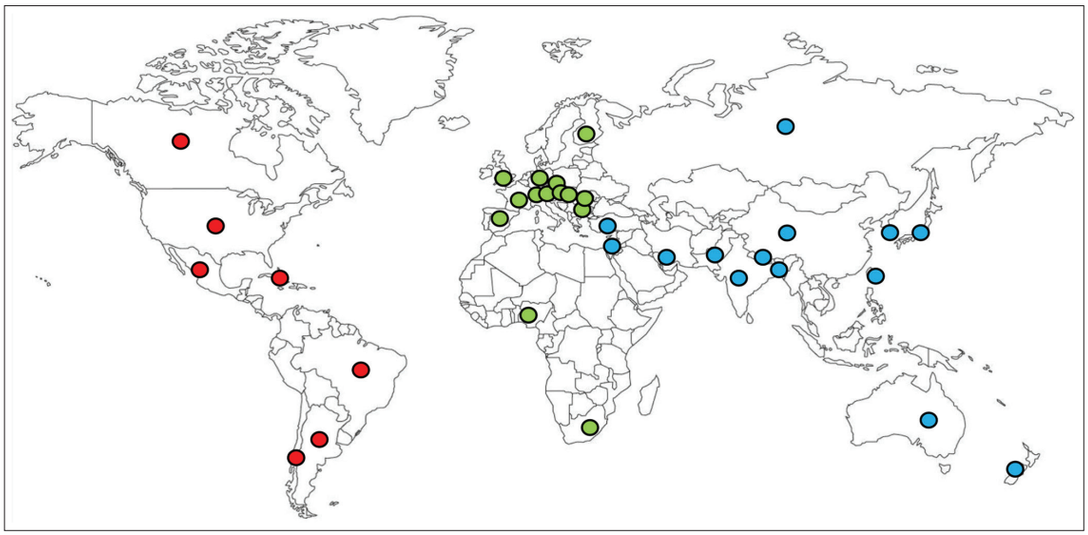

The geographical distribution of the responding IUPS member organizations.
IUPS Americas (red circles): Canada, USA, Mexico, Cuba, Brazil, Argentina, Chile
IUPS Europe & Africa (green circles): Finland, UK, Germany, France, Spain, Hungary, Czech, Slovakia, Slovenia, Switzerland, Romania, Bulgaria, Nigeria, South-Africa
IUPS Asia & Oceania (blue circles): Russia, Turkey, Israel, Iran, Pakistan, India, Nepal, Bangladesh, China, Taiwan, South Korea, Japan, Australia, New-Zealand

strengthen physiology worldwide, including an emphasis on activities and technologies that promote physiology teaching and research and financial resources for such activities. For example, the Bangladesh Society of Physiologists stated that the "*IUPS can give more attention to the promotion of physiology education and research especially in under privileged developing countries.*" The Chinese Physiological Society in Taipei would like the IUPS to "*raise the awareness of basic research in women's health-related diseases.*"

The Pakistan Physiological society pointed out that "*in some countries (including ours) the basic medical sciences are a neglected entity, especially today when science is rapidly changing.*" They would like to see the IUPS "*launch a global projection of the Basic Life Sciences as foundation to all medical and life sciences.*" The American Physiological Society suggested broadening the cohort of invited participants at scientific meetings to include individuals "*irrespective of their self-identification as physiologists.*" This approach for an interdisciplinary meeting by reaching out to other basic scientists could serve to enhance the profile of physiology globally and perhaps increase membership in physiological societies.

The Chinese Association for Physiological Sciences specifically requested help in their further development and "*expanding the impact of CAPS in IUPS.*" The Physiological Society of India did not make a specific recommendation to the IUPS, but they provided an interesting profile about the teaching of physiology in their country. They sadly reported that "*basic physiologists (MSc, PhD under the Faculty of Science) are not allowed to teach in Medical Colleges as per norms of Medical Council of India.*" They added that "*Physiology as a basic science subject is taught in many colleges and universities in India for more than 100 years, but it is yet to be included in the list of UGC approved subjects, unlike its allied life science subjects.*"

Recommendations of the BGA to Strengthen the Global Physiological Community

At the General Assembly of the XXXVIII IUPS Congress held in Rio de Janeiro in August 2017, the BGA presented the first global report on Physiological Sciences, *Physiology Current Trends, and Future Challenges*. In this report, members had developed a set of recommendations to strengthen the global physiological community. While these recommendations may not be universally applicable, it began with the hope that societies would work together with the new Regional Representatives to usher a new wave of understanding and togetherness in the physiological community. In the second global report on *Physiology: Challenges and the Way Forward*, the members of the BGA have analyzed (a) how the challenges and recommendations have begun to be addressed by societies and physiologists, (b) the realization of the Physiome Project and Virtual Physiological Human: Applications in the Health Care Needs of Society, and (c) the generation of a road map in the fields of clinical, translational, and regenerative medicine. We opine that the seven recommendations from the 2017 Report still hold true for strengthening physiology and physiological sciences towards achieving "Health for All."

The world is now facing an unprecedented crisis in the form of a pandemic arising from the SARS-CoV-2 virus, and physiologists are working at the forefront to tackle this enormous crisis to understand the basis for the viral-induced significant disruption in homeostasis. This is an opportune time for physiologists to work with ecologists, biochemists, immunologists, pharmacologists, epidemiologists, and health-care personnel to limit the rapid, global viral spread to develop safe and effective drugs and vaccines, to conduct animal and clinical trials that provide common sense approaches to avoid the viral load, and to raise our concerns for "planetary health" that focuses on the wellbeing of humans and other living things within the entire ecosystem. This has motivated the formulation of an 8th recommendation.

Report From IUPS Council – Regional Representatives

While preparing for the 2022 IUPS-BGA Report, Jayasree Sengupta and Susan M. Barman, Chair and Vice Chair of the BGA, invited the IUPS Regional Representatives to submit a short report on activities, initiatives, or programs launched by their regional physiological societies that address the set of recommendations brought forth by the 2017 Report. It has been the hope that national societies work together with the Regional Representatives to usher a new wave of understanding and togetherness in the physiological community. The following items were received in response to the request.

From: Professor Vagner Antunes, IUPS Council Regional Representative - North/South America

It's my pleasure to share a brief report of perspectives about activities that could foster physiology worldwide.

One of the major concerns that I have discussed within the community of physiologists is related to the future of the young scientists. Speaking specifically of Brazil, but certainly this concern extends to other countries on the American continent, is the prospect of the young scientist to get a permanent job at a research institute. This has become a faraway dream and creates a terrible atmosphere of discouragement to pursue a career in science.

This is, in part, a consequence of an economic recession and a lack of investment in science and technology (current Brazilian condition). In this sense, my idea is to work together with the authorities of other scientific societies to foment projects and request financial support from research funding agencies to facilitate students' mobility among countries. This action can give young scientists the opportunity to have an international experience and become more competitive to search for a permanent job and share their expertise with others to promote science in both the research field and education. In this sense, the IUPS brand would provide great support for this project to be successful.

In this same line of reasoning, I think that the participation of the BRICS in physiology can play a fundamental role in this process of supporting the next generation of scientists. Our meeting in Saint Petersburg with the BRICS in 2019 was very productive. It is very important to reactivate the conversations to shape and make an appeal to the authorities of each country to promote the exchange of students and young scientists overseas.

Last but not least, another idea that I have is to bring IUPS closer to the America's scientific societies (mainly Latin America) of physiology through its active participation in

scientific events promoted by each society. In this sense, an initiative will be carried out by Sociedade Brasileira de Fisiologia (Brazilian Society of Physiology) at its next Annual Congress of Physiology. We plan to invite the President of IUPS to give a lecture to the Brazilian physiologists (young investigators as the main target) to show them how important IUPS is in the context of physiology at a global sense and encourage them to promote the physiological sciences with active participation in scientific events organized by IUPS.

These are my main ideas of how to contribute to the IUPS to foster the physiological sciences all over the world.

From: Professor Soga Sofola, President of AAPS and IUPS Council Regional Representative - At Large

Following the last IUPS meeting in Rio, Brazil August 1-5, 2017, the African Association of Physiological Sciences (AAPS) decided to organize an Educational Workshop and a scientific meeting in Kigali, Rwanda. The idea was to find a centrally situated country to allow increased attendance from across the African continent. Previously, most AAPS Conferences were dominated by four countries: Egypt, Nigeria, South Africa, and Sudan. In contrast, the Rwanda Workshop in 2018 had attendance from eight other countries: Benin Republic, Ethiopia, Kenya, Rwanda, Tanzania, Uganda, Zambia, and Zimbabwe. We had hoped to attract our Francophone colleagues, but only one from the Republic of Benin attended; the proposed attendee from Tunisia could not come. The total attendance was 77 participants, with five international speakers from Japan and USA. Professor Rob Carroll, Chair IUPS Physiology Education Committee and Professor Tony Macknight and his team from ADInstruments organized the Physiology Teaching Workshop.

AAPS was to have its 4th yearly Congress in 2020, but it was postponed due to the COVID pandemic. The AAPS meeting was held virtually from September 12 - 15, 2021 in South Africa, in collaboration with the Physiological Society of South Africa (PSSA) (https://www.facebook.com/aaps.africa/). AAPS organized four well-subscribed webinars between July and October 2020.

We tried to reach out to other national societies, especially our Francophone colleagues. We had a close contact with the delegate from Benin Republic, Professor Yessoufou who organized the francophone African Society of Physiology and Pathophysiology conference in June 2021. I gave a talk on 'Salt Intake and Blood Pressure, the African Perspective' to a mainly Francophone audience. We hope that this will attract more Francophone interest in the AAPS. We are also working assiduously to start regional societies such as West Africa as well as East and Central African Societies. This is still a 'Work in Progress' and should be encouraged.

Finally, preparations are on full throttle towards the AAPS Congress at the University of Witwatersrand, South Africa. Due to COVID pandemic, it will be a virtual meeting instead of the proposed hybrid format. We are looking forward to this meeting after the pandemic disruptions. We hope that more nations join us at this AAPS meeting to expand the reach of the society to our colleagues in more African countries. We are grateful for a strong secretariat that is supported by ADInstruments.

From: Professor Xiaomin Wang, President of FAOPS and IUPS Council Regional Representative -Asia/Oceania

The most visible recent activity in this region would be the 9th Congress of the Federation of Asian and Oceanian Physiological Societies (FAOPS 2019) that was held with a main theme 'Physiology of Life: Function and Mechanisms' in Kobe, Japan in March 2019. The first "Review of FAOPS" e-book was released at the conference to celebrate 30th anniversary of FAOPS. Those who contributed to the establishment and development of FAOPS were honored with the Lifetime Contribution Award at the meeting.

FAOPS was founded under the Chairmanship of Professor Masao Ito in November, 1990 in New Delhi, India as a unique organization comprising of countries located in Oceania region and across the Asia Continent. The goals were to promote the development of physiological sciences; strengthen exchanges in the physiological sciences and related disciplines and the popularization of knowledge of physiological sciences; to encourage physiological science research, and to promote all other local development of this branch of science and medicine. The Federation has 2 kinds of members, that is official (regular) members and associate ones. Nowadays there is officially 13 members from Australia, China, Chinese Taipei, Japan, India, Thailand, New Zealand, Iran, Israel, Korea, Malaysia, Philippine, United Arab Emirate and 6 associate members from Indonesia, Myanmar, Sri Lanka, Pakistan, Vietnam and Uzbekistan.

The original plan was to hold FAOPS 2023 in Iran and FAOPS 2027 in Korea. Due to recent changes in the politics/economy, FAOPS carefully consulted with Iranian Society of Physiology and Pharmacology (ISPP) and Korean Physiological Society (KPS).

The final decision made at the FAOPS Council meeting was to hold FAOPS 2023 Congress in Korea to be hosted by KPS, and to hold the FAOPS 2027 Congress in Iran to be hosted by ISPP.

Several other international and domestic events have been held in the region to promote physiology education, including a Physiology Quiz, in Pakistan, Japan, Mongolia, Philippines, Australia and Indonesia.

There are several dilemmas/challenges facing FAOPS's survival and development. Many countries find it difficult to join FAOPS, mainly due to their economy, education, science and technology, culture, politics, war, and other factors affecting national strength. Most of these countries are poor, and some countries in West Asia have been at war for years. The academic dominance of traditional physiology has gradually diminished. Physiology had a reputation as the mother of life sciences at the beginning of the 19th century, and the Nobel Prize in Life Sciences was named the "Physiology or Medicine Prize." Neuroscience, immunology, molecular biology, cell biology, pharmacology. and biophysics disciplines were booming; but now there are too few young scholars and excellent teams in physiology. New technologies and concepts are emerging, such as omics, big data, AI, 3D printing, and brain-machine dialogue. It is important for physiology to break through the original boundaries to foster multidisciplinary cross-integration and incorporate the new technologies into the teaching and scientific research content of physiology, system physiology, or integrated biology so that physiology re-emerges as a bright field. These issues are not just FAOPS challenges, but more global concerns. They deserve attention and discussion about what we should do at this critical time in history.

From: Susan Wray, First Vice President, IUPS on behalf of IUPS Council Member and Regional Representative for Europe

Since the IUPS congress in Brazil the world has suffered the shock of a pandemic caused by the SARS-CoV-2 virus.The response to this and its effects has dominated the agenda of physiologists throughout Europe. The Federation of European Physiological Societies – FEPS- and national societies have been significantly challenged, with meetings cancelled and normal international collaborations and exchanges paused.

For FEPS, of which I serve as President, we realized that this was a period where increased communication with members was necessary, as well as forming stronger strategic and political alliances with the overarching aim of strengthening physiology in Europe and beyond.

In recent regional meetings with groups of the 30 national societies that make up FEPS, similar stories were told. If one word could sum up these discussions, it would be "resilience". Scientific meetings were cancelled, but then transitioned the next year to virtual meetings, and some this year have moved to hybrid meetings. We have all become expert Zoom technicians!Labs were closed throughout the continent and the impact of this on productivity and early career stage physiologists, cannot be downplayed. Physiologists were however resourceful and creative, and many caught up with their pipeline of data analysis and paper writing, followed by reviews and grant writing when the data ran out. Teaching of physiology became almost entirely virtual, and suggestions for how to continue practical labs in new forms, shared. There are still increased financial pressures on funding for both grants and career opportunities for early career researchers. Within the UK major funders such as the Wellcome Trust worked with scientists to adapt deadlines and criteria and showed flexibility.

Local and European-wide analyses have shown that the impact of the pandemic on female scientists has been more severe than on their male counterparts, as, in general, they juggled home schooling and caring responsibilities, along with increased teaching and pastoral duties. Following events in America that brought the Black Lives Matter campaign to worldwide prominence, many national societies and FEPS reviewed their inclusivity and put greater emphasis and effort into the Equality, Diversity, and Inclusivity agenda.

The Europhysiology2020 meeting was cancelled, and with it a planned IUPS/FEPS workshop on Africa-European physiological collaborations. This should now occur at Europhysiology2022 in Copenhagen.

Finally, I will mention *The Tribute of Physiology for the understanding of Covid19. Disease*. This was edited by Georges Leftheriotis of France and others, including the former IUPS President, Denis Noble.I think this collection of work clearly shows how vital understanding organs, and their interconnections with other tissues and systems, i.e. physiology (!), is to understanding and combating diseases. It is freely available here:

https://www.frontiersin.org/research-topics/14265/the-tribute-of-physiology-for-the-understanding-of-covid-19-disease

Challenges in Physiology and the Way Forward

Research, a complex multi-dimensional endeavor, occupies a central place in pushing the borders of knowledge and understanding of physiology and pathophysiology for the benefit of mankind. The geophysical, political, and economic foundations are hugely varied across the globe; and they help to determine how physiology can make progress under the respective social and legislative environments. Ideally, a country should provide its research base strong support through competitive funding, effective collaboration to share knowledge, and technical expertise for academic and industrial efforts in tandem. Developed and developing nations could work together in a region-specific manner to promote development of research infrastructure and transfer of expertise. There is an urgent need for innovative development of *in vivo* and *in vitro* experimental models, *in silico* models and the training of young scientists in these fields. There is also a need to encourage national and international research collaborations to create an effective functioning research system that will benefit the wider community. The recognition of physiology education and research by engineering institutions and collaborations would help in the promotion of biomedical engineering, the growth of regenerative medicine, and the development of medical devices. The questions provided to IUPS member organizations were intended to gather information covering multiple aspects linked to the global conduct of research and education in physiological sciences across the physiological societies. Societies from the following regions have provided specific relevant information for this report: Argentina, Australia, Bangladesh, Brazil, Bulgaria, Canada, Chile, China, Chinese Taipei, Cuba, Czech Republic, Finland, France, Germany, Hungary, India, Iran, Israel, Japan, Korea, Mexico, Nepal, New Zealand, Nigeria, Pakistan, Russia, Romania, Slovakia, Slovenia, South Africa, Spain, Switzerland, Turkey, the UK, and the USA.

Academic Resources

Understanding of physiology and physiological practice is the backbone of medical sciences. It is concept-based, and with the advent of newer biophysical, cellular, and molecular biology tools we are beginning to realize the complexity of multi-state, multi-level operations in the functioning of living cells and organisms. Sir William Osler (1849–1919) is regarded as *the Father of Modern Medicine* and helped to establish the Johns Hopkins University School of Medicine. He is the author of *The Principles and Practice of Medicine* (1892) in which he wrote "*Á man cannot become a competent surgeon without the full knowledge of human anatomy and physiology, and the physician without physiology and chemistry flounders along in an aimless fashion, never able to gain any accurate conception of disease, practicing a sort of popgun pharmacy, hitting now the malady and again the patient, he himself not knowing which.*" Osler expressed particularly the relationship between basic sciences and clinical medicine and considered that diseases may be viewed as "*experiments of nature*" that may reveal unknown or unappreciated physiological mechanisms the investigations of which would advance our fundamental biomedical knowledge (Hammer GD and McPhee SJ, *Pathophysiology of Disease: An Introduction to Clinical Medicine*, McGraw Hill, 2014). Understanding the principles of physiology cannot be achieved solely through rote learning from regular didactic lectures; the use of "flipped classroom" modules, team-based learning, case-based learning tools, and problem-based learning modes is to be encouraged. The vertical model of physiology that starts at the basic level with upward integration in the clinical sciences should be promoted for creating healthy collaboration between physiologists and the clinical faculty.

Collaborative Efforts and the Creation of Global Networks

Physiology without borders: Report on physiology education workshops in India - IUPS initiatives (2018–2019)

A group of physiologists from India and across the globe took cognizance specifically of three out of seven recommendations of the first global report of Physiology that was placed by the IUPS in 2017, **Physiology – Current Challenges and Future Trends:** "*Networks and working groups should be created, domestically and internationally, by IUPS and member societies to facilitate the exchange of knowledge and best practice in teaching and research*" (Recommendation 2).

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"*Societies should continue the efforts of the IUPS Outreach Programme to increase support among physiologists for IUPS initiatives and furthering of the World Health Organization's Health for All agenda*" (Recommendation 3). And "*Societies should develop resources to improve the teaching and learning of physiology, and to ensure graduates have a full appreciation of the complexities at all scales of physiological understanding*" (Recommendation 5). A Tri Series Physiology Education Workshop as an IUPS Initiative chaired by D. Ghosh, All India Institute of Medical Sciences, India was conducted in different regions of India in 2018 (November 3–18) and one Workshop in 2019 (November 5–7). These workshops traced the spirit behind these recommendations which bore the spirit of physiology *sans border* to release the large capacity of liberalism inherently present in the practice of teaching-learning of physiological sciences because of its multi-dimensionality and scalability. The workshops aimed to hold discourse on the theory and practice of infusing interactive, evolutive, and adaptive conditions in the teaching-learning of physiology; make the physiology teachers aware of the primary role of brainstorming, discussion, coaching, and facilitating students' learning, attitude, and aptitude; facilitate the exchange of knowledge about best practices in physiology teaching; improve the quality of physiology teaching through creating networks and working groups by training teachers of basic sciences, medicine, veterinary, and dentistry at national and international levels. Awareness and "*hands on*" experience about the true potentiality of case based learning (CBL), problem based learning (PBL) and flipped class room (FCR) models achieved by 126 faculty teachers in physiology – basic and medical sciences, veterinary, and clinical sciences, nursing, and paramedical field – through creating networks and working groups at national and international levels to facilitate the exchange of knowledge about best practices in physiology teaching as innovative tools for physiology education. The faculty members who formed the panel of experts for these workshops included scientists and teachers of physiology from Finland, India, Malaysia, Taiwan, and USA [Table 7]. The funding support was received from the ICMR, Government of India and the IUPS-Education Committee for the conduct of the tri-series "Physiology Education Workshop" in 2018, and from the Dr. Ramdas Pai and Mrs. Vasanthi Pai Endowment Fund of SMU, and the IUPS-Education Committee for the workshop on "Medical Teaching Methodologies" in 2019.

Reports of the workshops can be found at:

https://www.iups.org/report-on-iups-hands-on-workshop-on-medical-teaching-methodologies-at-smims-smu/

Physiology Education Workshop: AIIMS-Jodhpur, 2018. [1] Inauguration in presence of Dean, AIIMS-Jodhpur, Julie Chan, President, IUPS and D. Ghosh, Convenor; [2] Faculty, Organizers and Participants; [3-5] "Hands on" CBL [3], PBL [4] and FCR [5] sessions; [6] Memento presentation by Dean, AIIMS-Jodhpur to President, IUPS.

Table 7. Faculty of the Physiology Education Workshops in India - IUPS Initiatives (2018–2019).

Name	Affiliation
S. Barman	Vice Chairperson, BGA-IUPS; Professor, Pharmacology and Toxicology, Michigan State University, Michigan, USA
B. Bhandari Rathore	Associate Professor, Physiology, Government Institute of Medical Sciences, Greater Noida, Uttar Pradesh, India
M. Bhattacharjee	Professor, Physiology, Vardhaman Mahavir Medical College-Safdarjung Hospital, New Delhi, India
J. Chan	President, IUPS; Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan
S. H. H. Chan	Director and Distinguished Chair, Professor, Institute for Translational Research in Biomedicine at Chang Gung Memorial Hospital, Kaohsiung, Taiwan
D. Chandran	Associate Professor, Physiology, All India Institute of Medical Sciences, New Delhi, India
R. Carroll	Chairperson, IUPS-Education Committee; Professor, Physiology, Brody School of Medicine, East Carolina University, North Carolina, USA
S. Das	Associate Professor, SHKM Medical College, Nalhar, Haryana, India
D. Ghosh	Chairperson, Physiology Education and Teaching Methodology Workshops; Professor, Physiology, All India Institute of Medical Sciences, New Delhi, India
S. Ghosh	Associate Professor, Centre for Education, International Medical University, Kuala Lumpur, Malaysia
M. Kaur	Assistant Professor, Physiology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India
T. Macknight	Professor, Director, Co-Founder & Scientific Consultant, AD Instruments, New Zealand
S. P. Muthukrishnan	Assistant Professor, Physiology, All India Institute of Medical Sciences, New Delhi, India
L. Peltonen	Secretary, Finnish Physiological Society; Professor, Biomedicine and Physiology University of Helsinki, Biomedicum, University of Helsinki, Finland
J. Sengupta	Chairperson, BGA-IUPS; Former Chairperson, Physiology, All India Institute of Medical Sciences, New Delhi, India
R. Sharma	Professor, Physiology, Vardhaman Mahavir Medical College-Safdarjung Hospital, New Delhi, India

Chandran DS, Muthukrishnan SP, Barman SM, Peltonen LM, Ghosh S, Sharma R, *et al.* Physiology without borders: report on physiology education workshops in India-IUPS Initiatives (2018-2019). Adv Physiol Educ 2020;44:309-13. doi: 10.1152/advan.00050.2020.

Chandran DS, Muthukrishnan SP, Barman SM, Peltonen LM, Ghosh S, Sharma R, *et al.* IUPS physiology education workshop series in India: Organizational mechanics, outcomes and lessons. Adv Physiol Educ 2020;44:709-21. doi: 10.1152/advan.00128.2020.

Chandran DS, Muthukrishnan SP, Barman SM, Peltonen LM, Ghosh S, Sharma R, *et al.* Operations perspective to competency-based medical education (CBME): Experiences of IUPS-ICMR physiology education workshop series (2018-2019) conducted in India. Indian J Med Res 2021 (In Press).

Physiology Education in Africa and other Resource-Challenged Regions: Reflections on Current Practices and Charting the Way Forward: Workshop hosted by the University of Rwanda, December 2-4, 2018

The African Association of Physiological Sciences (AAPS) held an Education Workshop, titled "*Physiology Education in Africa*

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Physiology Education Workshop 2018: NEIGRIHMS, Shillong. [1] Inaugural Lamp Lighting; [2] Faculty, Organizers and Participants; [3] FCR 'hands on' session; [4] Observer's comments by Moloy Mandol; [5] Valedictory Lecture by Liisa Peltonen; [6] Certification by Susan Barman to Organizing Secretary, Rubi Dey; [7] Cultural Program by the undergraduate students of NEIGRIHMS at the Valedictory event.

Physiology Education Workshop 2018: GMC, Kozhikode. [1] Inaugural Lecture by Robert Carroll; [2] Faculty, Organizers and Participants; [3] FCR "hands on" session; [4] Plenary Lecture by Dinu Chandran; [5] Plenary Lecture by M. Bhattacharjee; [6] Certification by Convener, D. Ghosh to a Participant; [7] Memento presentation by Organizing Secretary, K. Kalyanikutty to J. Sengupta; [8] "Harvest Dance" by undergraduate students of GMC-Kozhikode.

and other Resource Challenged Regions – Reflections on Current Practices and Charting the Way Forward," from December 2-4, 2018, in Kigali, Rwanda. The choice of Rwanda was based on the conscious need to select a centrally located African Country to increase participation. Previous AAPS conferences had mainly involved four countries – Egypt, Nigeria, South Africa and

Medical Teaching Methodologies 2019: SMU-SMIMS, Gangtok, Sikkim. [1] Inaugural session, Vice Chancellor-SMU and Convenor, D. Ghosh; [2] Faculty, Organizers and Participants; [3] Plenary Lecture by Susan Barman; [4,5] "Hands on" sessions on CBL [4], and PBL [5]; [6] Student Symposium; [7-9] Panel Discussion on CBME: Panelist, F. A. Zaman [7], Chairman D. Ghosh with the panelists: M. Kaur, A. Mishra, S. P. Muthukrishnan, F.A. Zaman, R. Sharma and M. Bhattacharjee [8], Certification by J. Sengupta [9].

Sudan. The AAPS Workshop held in Rwanda had seventy (70) participants joining from 11 countries - Benin Republic, Egypt, Ethiopia, Kenya, Nigeria, Rwanda, South Africa, Sudan, Tanzania, Zambia, and Zimbabwe. The Workshop was attended by J. Chan, President, IUPS, R. Carroll, Chair of the IUPS Education Committee, G. Sieck, D. Silverthorn from USA, N. Kiobuchi from Japan and S. Chan from Taiwan.

The Workshop began on December 1st with Practical Teaching Methods organized by T. Macknight and his team from ADInstruments that provided "hands-on" practice in the use of KuraCloud and the PowerLab/Lab Tutor. The AAPS Workshop had organized Plenary lectures as well as Symposia and Discussion groups that included: Assessment Methods, Curriculum Design, Novel Teaching Methods including ICT, Evaluation, Design of Practical Classes as well as discussions on Enhancing Networking and Mobility among African Post Graduate Students. The Workshop included poster presentations on Physiology Education. As part of the requirement from The Physiological Society there was a good gender mix of presenters, session chairs and rapporteurs. A brainstorming session was held on enhancing the profile of the *Journal of African Association of Physiological Sciences*.

The Vice Chancellor, University of Rwanda, P. Cotton and B. Gahutu, Chair of the Local Organizing Committee provided the venue and logistics. The Workshop was supported with funds received from several agencies that included ADInstruments, The Physiological Society, The IUPS Education Committee, the Welcome Trust, Axiology Labs, Canadian Physiological Society,

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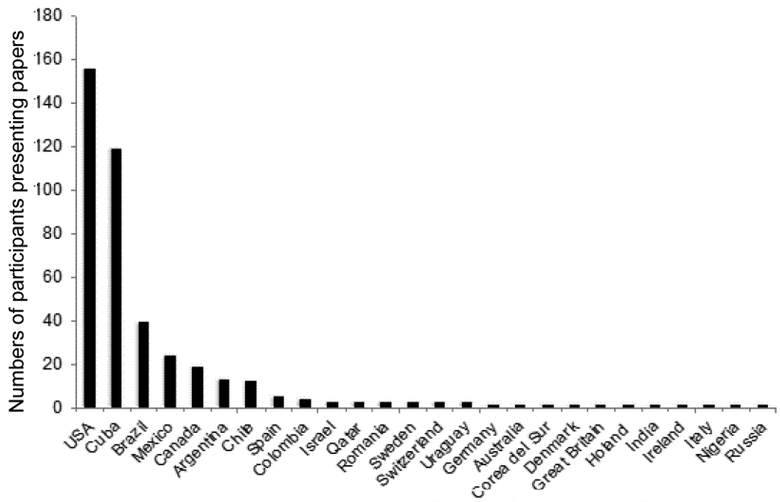

Global distribution of attendees at the Second Pan-American Congress of Physiological Sciences, May 27-31, 2019, Havana, Cuba

AAPS Workshop on Physiology Education in Africa and other Resource-Challenged Regions, 2018. [1] Inaugural Lecture by D. Silverthorn; [2] Session being attended by Y. El-Wazir, G. Sieck, S. Chan, J. Chan; [3] Faculty: M.F. Essop, IUPS President, J. Chan and S.O. Elias; [5] Faculty, Organizers and Delegates of the Workshop; [6] traditional Rwandan Dance performance.

Kessington Adebukunnola Adebutu Foundation of Nigeria as well as the Taiwan Physiological Society.

Second Pan-American Congress of Physiological Sciences, May 27-31, 2019, Havana, Cuba

The 2nd Pan-American Congress of Physiological Sciences held in Havana, Cuba, 2019 was chaired by A. Dorta-Contreras and G.C. Sieck. It was attended by 413 attendees from 26 countries with 314 programmed presentations. The program included 5 plenary speakers and 12 keynote speakers, all from the Americas; 31 symposia with 126 speakers and 3 workshops with 14 participants and 3 poster sessions with 157 poster presentations. The Congress was supported by the American Physiological Society and the IUPS.

BRICS (Brazil, Russia, India, China and South Africa) Symposium on Stress and Conference on Integrative Physiology at the Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia, September 23, 24, 2019

A Symposium on Stress was held at the Pavlov Institute of Physiology, Russian Academy of Sciences (RAS) in Saint Petersburg, Russia on September 23, 2019 in coordination with academicians of the BRICS nations: Brazil, Russia, India, China, and South Africa. It was chaired by L. Filaretova, Director, Pavlov Institute of Physiology and Chair, IUPS Commission III - Endocrine, Reproduction and Development. The BRICS Symposium was attended by J. Chan, President, IUPS; J Sengupta, Chair, Board of the General Assembly (BGA) of IUPS; V. Roberto Antunes, IUPS Regional Representative-North/South America; M. F. Essop, Member, BGA; S. Chan, Director, Institute for Translational Research in Biomedicine, Kaohsiung Chang Gung Memorial Hospital, Kaohshiung, Taiwan; and D. Ghosh, Head, Molecular Physiology Laboratory, Department of Physiology, All India Institute of Medical Sciences, New Delhi, India. The BRICS Symposium was attended by G. Baffy, VA Boston Healthcare System and Brigham Women's Hospital, Harvard Medical School, Boston, USA; D. Zelena, Institute of Experimental Medicine, Budapest, Hungary; and E. Savochkina, E. Rybnikova, Y. Shelepin, Y. Gerasimenko from the Pavlov Institute of Physiology, RAS, Saint Petersburg, Russia. J. Chan addressed that the primary purpose and basic foundation of the symposium was to realize one of the seven commitments the IUPS had made to the global community of physiologists, in *Physiology-Current Trends and Future Challenges* (2017): "*Networks and working groups should be created, domestically and internationally, by IUPS and member societies to facilitate the exchange of knowledge and best practice in teaching and research.*" It was a historic moment for the IUPS since it was the first time that scientists from BRICS had gathered at the Pavlov Institute in Russia to forge together a research network for understanding the integrative physiology of stress. (For details see: iups.org/report-of-iups-brics-symposium/.

Global collaborative networks established through the IUPS provide understanding on the impact of stress, and infertility in patients suffering from endometriosis. (Ghosh D, Filaretova L, Bharti J, Roy KK, Sharma JB, Sengupta J. Pathophysiological basis of endometriosis-linked stress associated with pain and infertility: A conceptual review. *Reprod Med* 1: 32-61, 2020. doi:10.3390/reprodmed1010004).

Conference on Integrative Physiology held at the Pavlov Institute of Physiology of the Russian Academy of Sciences at Saint Petersburg, Russia, September 24, 2019

The Russian Academy of Sciences at Saint Petersburg held a Conference on Integrative Physiology at the Pavlov Institute of Physiology on September 24, 2019 in honor of the 170th birth anniversary of Ivan Petrovich Pavlov. In her introduction, L. Filaretova discussed the role of Ivan Pavlov in creating the foundation of Integrative Physiology and for training a whole school of physiologists and psychologists who continued his ideas about integrative physiology and

behavior to future generations. In her inaugural address, J. Chan recalled that Ivan Pavlov had set the stage for physiological sciences in the 20th century for which he was awarded the Nobel Prize in Physiology or Medicine in 1904, becoming the first Russian Nobel laureate; he is also remembered for his immense contributions in psychology. The conference was attended by the international delegates, J Chan, J. Sengupta, V. Antunes, M.F. Essop, S. Chan, D. Ghosh, G. Baffy, D. Zelena, I. Zhdanova, I. Abraham and members of the Russian Physiological Society, faculty and students of the Pavlov Institute of Physiology. (For details see:
https://www.iups.org/
report-on-russian-conference-on-integrative-physiology/

Resources in Learning and Research

Study of physiology as a subject in its own right may be expanded to create the nexus that exists between physiology, molecular biology, environmental biology, ecology, genetics, and systems biology. There is a need to emphasize the importance of understanding basic and integrative physiology in the underpinning of clinical practice. This may primarily be attained through well-designed undergraduate and postgraduate courses that medical students may also optionally attend. The American Physiological Society provides academic resources by offering opportunities for graduate students to participate in the annual meeting at Experimental Biology and in smaller meetings and through web based resources available to teachers at all levels, including the development of guidelines for physiology teaching curricula at medical schools. The Local Undergraduate Research Award in Physiology (LURAP) is an initiative of the American Physiological Society to recognize undergraduate student activities in research. The Argentinean Physiological Society with support received from the IUPS organized two symposia at the Pan-American Congress in Cuba in 2019; one was a research symposium and the other one was a teaching symposium on how to motivate the students to learn physiology. *Physiological Mini-reviews*, an official journal of the Argentinean Physiological Society, started publishing in 2019 and is the journal of the "new" Latin America Society.

The Bangladesh Physiological Society organized workshops for updating teaching methodology or curriculum review, but mostly these occur at the institutional level by the Medical Education Units in University and/or medical colleges. The

Conference on Integrative Physiology held at the Pavlov Institute of Physiology of the RAS at Saint Petersburg, Russia, September 24, 2019. [1] Inauguration; [2-10] Speakers: (2) J. Chan, President, IUPS; (3) M.F. Essop, Member, BGA-IUPS; (4) V. Antunes, Council Member, IUPS; (5) D. Zelena, Hungary; (6) G. Baffy, USA; (7) I. Zhdanova, USA; (8) S. Chan, Taiwan; (9) J. Sengupta, Chair-BGA-IUPS; (10) I. Abraham, Hungary; [11] Distinguished Guests, Speakers and Delegates.

society plays a moderate role in facilitating research by organizing periodic seminars on current research activities in Physiology. Promotion of academic resources by the Brazilian Physiological Society includes the organization of regional meetings with multidisciplinary activities, new political investment in science and education, and discussions of scientific integrity. Each year the Sofia branch of the Bulgarian Physiological Society organizes workshops with participation of Medicine and Biology students. Despite being a small society with limited capacity to implement substantial training resources, in 2017 the Canadian Physiological Society re-initiated its annual national-scale events to highlight physiology research in Canada. Such meetings prioritize trainee participation by including opportunities to present, awards, travel support, and career mentorship. The Chilean Physiological Society participated in the PanAm II Congress of Physiological Sciences held in Cuba in 2019 with support from the IUPS. Chile will be the venue of the PanAm III Congress of Physiological Sciences in 2023. The Chinese Association for Physiological Sciences is preparing for the IUPS 2022 Congress and organized *Frontiers of Physiology Forum* that was held on October 30, 2019 at the Peking University Health Science Centre, Beijing. Academic resources in physiology are strengthened by Chinese Association for Physiological Sciences through the development of a System Based Integration Curriculum that includes problem-based learning tutorials, Comprehensive Experiments (Problem Based), videos, and Massive Open Online Courses (MOOCs).

In Cuba the study of physiology was previously joint with morphology but beginning 2 years ago, physiology is taught as a separate subject in the curriculum of medicine, dentistry, and technology. The Cuban Society of Physiological Sciences has incorporated a Cuban Movement (*Alumnos-ayudantes*) that involves the participation of outstanding medical students in teaching as Assistant Students who work in close contact with their professors to help with teaching and in research labs. It is an important contribution because the mentor-mentee relationship becomes a strong link with good results in teaching and research and in attention to vocational orientation and community work. Scientific Meetings are organized annually by university students with the support from the medical universities and the best papers selected for presentation in a National Scientific Student Forum. The Teaching Committee with representatives from all physiology departments at medical faculties in the Czech Republic work to introduce new teaching methods, computer-aided learning tools, simulators, and educational films.

The Finnish Physiological Society reports that the National Network of Medical Schools under the auspices of the Ministry of Culture and Education in Finland has invested for digitalization, harmonization, and modernization of teaching of all medical sciences since 2017. The MEDigi Project and the "*Finnish Doctor – Outcomes for Graduates*" involves members of the Finnish Physiological Society who have produced digital material for the future "national digital campus" together with the other national operators (https://www.medigi.fi/en/home-page.html); the language is Finnish and Swedish. The Medical Faculty of the University of Helsinki is leading this project whose goal is to create National Outcomes by national collaboration. The working methods of the network or group include the so-called Delphi method, which has been used within different fields of research and includes a panel of experts who gives their responses anonymously. The objective of the national team of about 120 persons is to assess the important graduating doctors' learning outcomes, based on demands of the Finnish health-care system and international guidelines. The evaluation takes place with three separate rounds during which the learning outcomes are developed on the basis of the estimates and proposals given by the expert panel.

The French Society of Physiology has developed communication and interaction with other domestic national societies such as the National Nephrology Society, the French Society of Nutrition, and the National Society of Neurophysiology. They have proposed a homogenous teaching curriculum in physiology at the national level with the launch of the *Collège Français des Enseignants Universitaires de Physiologie en Santé*. A Continued Medical Education (CME) program in the field of "*Experimental Physiology on animal models*" is an indispensable tool for a career as a physiologist with accreditation by the National Education Programme. With the help of its different disciplinary sub-groups, the French Physiological Society published a text book, entitled "*Physiologie Humaine*" (Editor: Hervé Guenard) as part of the Masson Editions/publications series for medical students; they have also launched "*Physiome, the NewsLetter*" published every 6 months and distributed free of cost to all members. The Hungarian Physiological Society reports that heads of

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the physiology departments regularly meet and exchange material and methods in teaching of physiology; also there is a tendency for harmonizing the assessment methods with a common databank for MCQ tests.

The Association of Physiologists and Pharmacologists of India has taken initiatives to create networks and working groups at a national level based on the core area of research and specific domains of clinical physiology. The Physiological Society of India through its website http://www.physiologicalsocietyofindia.org/communicates academic and scientific programs with its members throughout the country and abroad. Since 1960 the Society publishes affordable laboratory notebooks on Histology, Experimental Physiology, and Biochemistry for undergraduate students of colleges/universities where physiology is taught under the basic sciences. The Physiological Society of India also has published a quarterly journal, *Indian Journal of Physiology and Allied Sciences*, since 1946 using its own funding. The Iranian Society for Physiology and Pharmacology is geared toward increasing scientific collaborations among its researchers. It is also involved in the organization of International Brain Research Organization schools and international workshops and the conduct of refresher courses in Physiology and Pharmacology during their biannual Congresses. The Israeli Physiological Society organizes annual meetings and satellite meetings with exchange of methods, views, etc., usually on individual basis among society members. The Physiological Society of Japan promotes a Physiology Educator System at its annual meetings and publishes books for physiology learning: *Workbook of physiology for computer-based test (Jpn)*, *Textbook of Educational Laboratory Courses of Physiology (Jpn)*, and *Textbook of Anatomy and Physiology for National Nursing Examination (Jpn*).

The Mexican Society of Physiological Sciences encourages collaborations among its members. The Physiological Society of Nepal participates in the Inter-medical School Physiology Quiz and workshops on revising medical curriculum for improving undergraduate and postgraduate physiology teaching along with Programs for Continuing Professional Development. The Physiological Society of New Zealand runs a national meeting under the umbrella of the MedSciNZ to promote physiological research in New Zealand and to present awards to students and emerging and senior researchers. The Romanian Physiological Society is involved in the development of an e-learning environment (based on Moodle platform) where undergraduate students can access all lectures of human physiology and laboratory protocols with information being regularly updated in accordance with new physiology concepts. The Russian Academy of Sciences and Russian Physiological Society have had discussions of academic research with scholars from educational institutes. Physiological understanding is viewed to be complex but basic, necessary, and integrative for medicine. The complexity of physiological understanding is opposite to a simplistic reductionist approach and is regarded as a necessary part of understanding functioning of the organism. Specifically, behavioral researchers proved major deficiencies of conventional knockouts for interpretation of single gene function, etc.

The Slovak Physiological Society organizes yearly workshops as joint meetings with the Czech Physiological Society; the society also noted that academic resources are enriched by teaching physiology at Universities with programs in General Medicine, Dental Medicine, Natural Sciences, etc. and through PhD programs in Normal and Pathological Physiology at Universities and Academy of Sciences. The Slovenian Physiological Society holds workshops on recent advances in physiology for elementary and secondary school biology teachers. Workshops are organized on the day of the Nobel Prize in Physiology or Medicine; throughout the year invited talks are organized on recent advances in physiology that are open to all university teachers. The Spanish Society of Physiological Sciences website has a space for teaching content and physiological simulators. In their congresses, the Society conducts symposium dedicated to improving the teaching and learning of physiology. The Spanish Society of Physiological Sciences semi-annually publishes a scientific journal "*Fisiología*" ISSN: 1889-397X. The annual events of Life Science Switzerland and LS2-Physiology section co-organize yearly educational and scientific meetings with Germany, Austria, and other EU-countries. On-line courses on renal physiology developed within the NCCR-Kidney.CH network for PhD students and course materials in medical physiology are available for students on MOODLE.

The Chinese Physiological Society in Taipei organizes a retreat for junior faculties, post-doc and graduate students, as well as annual meetings and student exchange programs, a dual diploma program, and cross universities projects such as

Taiwan International Graduate Program-Academia Sinica and Joint Annual Conference of Biomedical Science, Symposium of Chinese Physiological Society for Research-Teaching Excellence, and Research Project Presentation of Ministry of Science and Technology. The Turkish Association of Physiological Sciences promotes improvements in teaching and learning of physiology that are accomplished mostly by the Medical Schools. The Physiological Society (UK) is involved with the IUPS to establish networks in areas such as Sport and Exercise Science.

Online courses and academic physiology resources created and maintained by physiological societies include those from the American Physiological Society, Chinese Association for Physiological Sciences, Czech Physiological Society, French Physiological Society, Life Science Switzerland LS2-Physiology, Physiological Society of India, Romanian Physiological Society, Spanish Society of Physiological Sciences, FPS, and The Physiological Society (UK) are provided in Table 8.

The Physiological Society of Pakistan (PSP) has expressed a serious concern in the development of academic resources in physiological sciences in Pakistan in view of the recent decisions by the government regarding regulation of medical education and medical practices in which the basic sciences are not considered integral part of medical education. The postgraduate qualifications are neither registered nor taken as a pre-requisite by Pakistan Medical Commission for career in basic sciences teaching. This change in policy has created unrest in the country, especially in those who desired to join basic sciences as teachers and researchers. The physiologists are not much encouraged to develop new tools and techniques in medical research under prevailing situation. The Physiological Sciences must be promoted at governmental as well as organizational level to keep pace with the rapidly growing research and development in the subject. This is possible only if the basic sciences in general and physiological sciences in particular are recognized as the foundation of medical and life sciences. A similar view has been expressed by physiologists from The Physiological Society of India as physiologists holding M.Sc/Ph.D under the faculty of Science are not allowed to teach in Medical Colleges as per the norms of the Medical Council of India.

Recommendations

Societies should develop resources to improve the teaching and learning of physiology, and to ensure graduates have a full appreciation of the complexities at all scales of physiological understanding.

Networks and working groups should be created, domestically and internationally, by IUPS and member societies to facilitate the exchange of knowledge and best practice in teaching and research.

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Porter Committee meeting in operation for selection of Physiology Development Fellowships to encourage diversity among students pursuing full-time studies towards a PhD in the physiological sciences and to encourage their participation in the American Physiological Society.

Networking and Mentor Mentee Programs

The opportunities for learning, teaching, and research in physiology are quite diverse across the globe. On the one hand, we find many scientific discoveries in physiological sciences and several well-run programs are being executed by The Physiological Society (UK) established in 1876. At the opposite end of the spectrum, the Bangladesh Society of Physiologists, a relatively new non-profit organization founded in 2006, is devoted to fostering education and research and dissemination of knowledge in medical physiology but has limited access to funds for academic resources and initiatives to strengthen the discipline of physiology. There is need to share scientific expertise to newly formed societies by the well-established international community, to identify opportunities for their development and progression, and to encourage confidence and autonomy in driving forward their goals in teaching and research in physiology. With the interconnectedness of modern science, the IUPS is in a position to bring together potential mentors and mentees who can share their experience and perspectives of working in different countries.

The Canadian Physiological Society has prioritized support and mentorship of trainees as a main component of its mission by including networking and mentorship activities at society-sponsored meetings and by offering travel support, awards, and presentation opportunities for trainees at society-affiliated meetings. Members of the Chilean Society of Physiological Sciences had mentors at The Physiological Society in the UK and will soon have person-to-person meetings to support the establishment of national and international collaborations. The Cuban Society of Physiological Sciences integrates in its program for medical science students the "*Alumnos- ayudantes*" as a strong movement in which medical students conduct research with team of physiologists in a Mentor/Mentee program. With the collaboration of the APS, Cuban members of the American Physiological Society are involved in the Local Undergraduate Research Award in Physiology to participate in Experimental Biology meetings in USA. The French Society of Physiology is involved in creating a sister societies like SA2P (Société Africaine de Physiologie et de Pathophysiologie, Senegal) and Moroccon Society of Physiologie, Rabat, Morocco. The Finnish Physiological Society promotes Mentor/Mentee relationships among physiologists within a specific field of physiological sciences, such as exercise physiology, endocrinology, and neuroscience. The organization of symposia, workshops, and science cafés by young researchers is facilitated by interactions with members of the Physiological Society of Japan and by organizing interdisciplinary symposia at annual meetings. Mentor-Mentee programs exist in Russia through "Scientific schools" as a system for promoting (including modest government financial support) engagement with large laboratories headed by an outstanding scientist with decades of successful research. Existence of a school implies scientists of various career stages can work in different Institutions, but follow a general direction of research introduced by the Founder. The Chinese Physiological Society in Taipei hosts a retreat for young investigators and post-docs and provides an opportunity for undergraduate students (biology major) from overseas to do an internship as part of an international summer research program.

A symposium was held at the PanAm II Congress in Havana in 2019 on "*Networking: Promoting Collaboration Across Borders*" co-chaired by G. Sieck and S. Barman as American Physiological Society members. Even though the attendance was not robust, in essence, it led to good interactions on the importance of mentoring to be successful. Furthermore, a message that came out clearly is "*that you never know what doors might be open to you by interacting with various role models who you meet along the way*." Various mentoring programs were discussed and while the attendees included more experienced individuals than junior scientists, they expressed interest in being a mentor and understood the importance of international mentoring programs. S. Barman, Vice Chair of the BGA-IUPS speaking at the Symposium highlighted that IUPS and BGA are taking an initiative to create a Mentor-Mentee Program to allow for the global progress of physiological sciences by:

* *Educating young PIs and faculty members in developing their research career*
* *Providing consultation/education to mentees from senior physiologists*
* *Creating a repository of mentors (members of BGA, IUPS Executive Committee, IUPS Commissions).*

S. Barman further highlighted a "Mentor-Mentee" initiative of the Indian Council of Medical Research (ICMR) as a National Workshop for *Advancement of Physiological Sciences* with

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Table 8: Online academic resources in physiology.

Member Organization	Online e-learning courses and academic resources
American Physiological Society	· Web based resources for teachers at all levels · Guidelines developed for physiology teaching curricula at medical schools
Chinese Association for Physiological Sciences	· System Based Integration Curriculum (including problem-based Learning tutorials) · Comprehensive Experiments (Problem Based) · Videos · MOOC
Czech Physiological Society	· Computer-aided learning and educational films · Simulators
Finnish Physiological Society	· National network of medical schools in Finland for digitalization, harmonization and modernization of teaching of all medical sciences since 2017, under the auspices of the Ministry of Culture and Education · National network for "Finnish Doctor – Outcomes for Graduates" (ongoing, all medical schools in Finland)
French Society of Physiology	· Launched Collège Français des Enseignants Universitaires de Physiologie en Santé to propose a homogenous teaching curriculum in physiology at the national level · Continued Medical Education program in "Experimental Physiology on Animal Models" with the accreditation by the National Education Programme
Life Science Switzerland (LS2)-Physiology	· Online courses on renal physiology developed within NCCR-Kidney.CH network · Courses in medical physiology available on MOODLE
Physiological Society of India	· Publishes laboratory notebooks since 1960 on Histology, Experimental Physiology and Biochemistry for undergraduate Physiology students in universities where this subject is taught under basic science · Notebooks updated/modified based on changes in the syllabus
Physiological Society of Japan	· Published books for physiology learning: *Workbook of physiology for computer-based test (Jpn), Textbook of educational laboratory courses of physiology (Jpn), Textbook of anatomy and physiology for national nursing examination (Jpn)*
Romanian Society of Physiology	· e-learning environment based on Moodle platform for undergraduate students with regular updating in accordance with new physiology concepts
Spanish Society of Physiological Sciences	· Website with teaching contents and physiological simulator · Publishes semi-annually a scientific journal *Fisiología* ISSN: 1889-397X
The Physiological Society	· MOOC program in Physiology widely available online

the support of the IUPS, chaired by J. Sengupta, Vice Chair of the BGA-IUPS in 2013 at three academic institutions in India. The Workshop had as mentors, senior physiologists who were members of the Physiological Society of India and the Association of Physiologists and Pharmacologists of India. The Workshop aimed to emphasize the need for reviving physiological sciences, to highlight the role of forums in creating research awareness in physiological sciences, and the important role played by mentors in shaping minds of young scientists to become great teachers and researchers in their respective fields. The Workshops aided young faculty members of physiology and life sciences to successfully build upon their research proposals based on consultations with respective mentors via teleconferencing and emails which upon

submission to the ICMR received grant funding for 3 years. According to the ICMR, eight research projects received from different regions of India were funded, six research projects completed and research studies are continuing in two projects.

In 2018, members of the BGA developed a proposal to conduct a Mentor-Mentee Workshop to help fulfill the recommendation of IUPS-BGA Report for the advancement of physiological sciences in countries of South Asia region. It was hoped to motivate similar activities in other parts of the world for the advancement of research in physiological sciences in a region-specific manner. It was proposed that the Program would proceed in four phases: (a) Selection of mentees based on their submitted project proposal from applicants through email; (b) face-to-face mentoring of selected projects of mentees at a Workshop by a panel of mentors; (c) set goals for follow-up meetings; (d) fine tuning of project through dialogue between mentor and mentee team through email/Skype; (e) review of progress being made; and (f) mentee to submit research proposal to funding agency (national/international) with inputs from his/her mentor. The planning of the program essentially followed the successful ICMR Mentor-Mentee program conducted in India in 2013, but the challenge remained in terms of securing funds for its execution as a global program.

Besides funds, another important challenge is in creating a "*Repository of Mentors*" volunteering to give their time/expertise for such a venture. S. Barman in this context emphasized that it remains critical to solicit mentees from geographical regions with experienced researchers as mentors; to recruit good mentors willing to devote adequate time to match both scientific and professional needs; and to have mentors trained for international collaborations.

Table 9 provides a preview of the likely advantages that a successful mentor-mentee program can provide.

The Indo-US Fellowship for Women in Science, Technology, Engineering, Mathematics and Medicine (STEMM) has created a new program through the Department of Science and Technology, Government of India. The Indo-US Science and Technology Forum jointly announced this new program that aims to:

* *Provide opportunity to bright Indian women students and scientists to gain exposure and access to world class research facilities in US academia and labs*
* *Promote research and capacity building for Indian women students and scientists in different frontline areas STEMM*
* *Pave way for the next generation of Women Scientists and Technologists from India to interact with American peers, thus helping to build long-term R and D linkages and collaborations*
* *Encourage, motivate and provide opportunity to outstanding women students to take up research path.*

https://iusstf.org/program/indo-us-fellowship-for-women-in-stemm

Table 9: Outcomes of successful mentor-mentee relationship*.

Career advancement and expanded thinking
Mentoring relationship allowed for exposure to new fields of enquiry and motivation to advance your career.
Increased scholarly confidence
Collaborative working
Mentee has freedom to share ideas, to get insights about work to be done, and to use mentor as a "sounding" board for brainstorming ideas.
Skills development
Opportunities to improve skills in areas of qualitative data interpretation and analysis; argumentation; and general academic writing skills
Goal setting and action planning

*Based on the presentation by S. Barman at the PAN AM II Congress of Physiological Sciences, Cuba, 2019

Recommendations

IUPS should oversee a new Global Mentorship Building Platform to facilitate Mentor/Mentee relationships among physiologists at various career stages, and in academic and clinical settings, to promote dialogue and aid career development.

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Outreach Programs

Several physiological societies adhered to the IUPS recommendations by offering Outreach Programs that give opportunities for increasing understanding on *what is physiology* among students, physiologists, clinicians, and the lay public.

The APS has a communications department which constantly develops press releases for public consumption. In addition, the APS has a platform for blogs such as *ISpyPhysiology* and *Dr Doolittle* to post routinely items of public interest. Numerous Twitter feeds and Facebook pages are aimed at the public. Bulgarian physiologists participate in the European Science Night "*Open Doors*" which offers activities for pupils, students, and the public that is organized by the Bulgarian Academy of Science. The Chilean Physiological Society organizes activities with direct approach to the Community. The Cuban Academy of Science helps in annual participation of exhibitions for children, young people, and the lay public; and the Cuban Physiology Society with the Cathedra of Scientific Communication have participated in the spread of physiology knowledge for the lay public and supported campaigns about hypertension and other disorders.

Popularization and promotion of the Czech Physiological Society on the internet targets the lay public with regular science-popularization activities in the media. The Physiological Society (UK) conducts annually a "*Physiology Friday*" as a celebration of physiology and its role in underpinning health. It also runs a MOOC program and will make the resources available on line shortly. The society awards grants and prizes to reward teaching excellence and support the development of best practice in higher education. It also awards smaller pots of funding for members to run outreach activities throughout the year, and especially on "*Physiology Friday*." It also provides funding for projects across the world and works on a lifelong health project. In Finland, physiologists as experts in their scientific fields are often seen in media, commenting on their own research and topical issues, or answering questions from the general public (e.g., science columns for children, articles in newspapers and magazines, and blogs on various topics). The *Finnish Science Barometer 2019* states that the vast majority of Finns eagerly follow science news and events. Citizens value research and trust in science's ability to solve societal and global problems. More reliance is placed on basic research than ever before. Scientific debate and contradictory research results are more tolerated by the lay public and not necessarily associated with the unreliability of science.

"*The Night of Science*" is a tradition undertaken by the Hungarian Physiological Society each year where several labs are open and available for visitors and methods are displayed; many scientific sessions are organized in the University cities of Budapest, Pecs, Debrecen, and Szeged. To create interest about physiology among school children in rural areas of West Bengal, the Physiological Society of India has an initiative to facilitate learning by not only undergraduate students but also the general public. The Physiological Society of Japan has recently initiated web-based programs to introduce to the public the research achievements and contributions of Physiological Society of Japan members. PSJ also organizes public lectures at annual meetings to promote the importance of physiological sciences. An annual activity of the Physiological Society of Nigeria uses the mass media to enlighten the public on the relevance of physiology in the society. Physiologists as members of the Slovak Physiological Society take part at all activities related to popularization of science (physiology) with Open Days for public at "*European Science Night*" and *Science and Research Week*." The Life Science Switzerland (LS2)-Physiology Society in Switzerland regularly encourages the public and the private sectors to sponsor *Foundation of Physiology* that is devoted to research by young physiologists. The *Scientifica* at UZH/ETHZ is a bi-annual forum to inform the public about research in general, but physiology is always present. The Chinese Physiological Society in Taipei with funding from the Taiwan government Ministry of Science and Technology (MoST) promotes "*Science Popularization Program.*"

Recommendations

Societies should continue the efforts of the IUPS Outreach Program to increase support among physiologists for IUPS initiatives and its furthering of the World Health Organization's Health for All agenda.

Societies should implement outreach activities to raise awareness of and interest in physiology among the public and encourage the uptake of physiology and related subjects by prospective undergraduate and postgraduate students.

Funding for Initiatives to Strengthen Physiology as a Discipline

The Undergraduate Summer Research Fellowship (UGSRF) program of the American Physiological Society recruits US and international undergraduate students who have had less than 9 months of prior laboratory research experience to complete a 10-week research experience in the laboratory of an established American Physiological Society investigator. Funds include a stipend, 2 years of complimentary American Physiological Society membership, and additional funds to attend and present at the Experimental Biology meeting. UGSRFs participate in hands-on research and learn to develop a hypothesis, design and troubleshoot experiments, collect and analyze data, and write and present results. In addition, these fellows have the opportunity to network with other undergraduate fellows conducting biomedical research, to explore the nature of research and the scientific process, to investigate physiology career options and what it takes to find career success, to learn about scientific writing and draft a meeting abstract, to learn about common ethical issues in figure and text preparation, and to pose their career questions to APS members. The Physiological Society (UK) offers research grants to physiologists less than 3 years into their first permanent academic position or returning to a permanent position after a career break. These are two examples of how societies help to strengthen physiology as a discipline. Most research in physiology is funded publically or philanthropically. The availability of funding varies widely around the world depending on economic strength and government priorities. Responding societies here describe the situation they experience in accessing funding to undertake research.

The Canadian Physiological Society supports activities primarily with the proceeds of a society endowment; it also receives support and sponsorship of the IUPS membership by the Canadian National Research Council. Funding from the APS to the Cuban Society of Physiological Sciences has facilitated development of the Quincke Research Scholarship annually for 3 years to allow medical students from ten countries to participate in the summer course held in Havana. Medical Education Cooperation with Cuba (MEDICC) a non-governmental organization based in Oakland, California, USA, also promotes US-Cuba health collaboration and highlights Cuba's public health approaches in its quest for health equity and universal health worldwide. Supported the rehabilitation of physiology basic sciences in 14 Cuban medical universities. The Cuban government supports research projects in physiology. In Finland, government funding (Ministry of Culture and Education, Finnish Science Academies) is received by the Finnish Physiological Society to support international initiatives. The Israeli Society of Physiology and Pharmacology receives donations from private sources for annual meetings that help to strengthen physiology as discipline as well promote collaborations. The *Journal of Physiological Sciences*, an official journal of the Physiological Society of Japan is supported by the governmental grant from Japan Society for the Promotion of Science. The Pakistan Science Foundation supports the Pakistan Physiological Society for conducting biennial Conference with donations from the host Institute. The National Research Foundation and the Medical Research Council funds research workshops for undergraduate students for the Physiological Society of Southern Africa. The Life Science Switzerland (LS2)-Physiology regularly receives support from the Swiss Academy of Science and the private sector to support educational events. Some universities (e.g., University of Zürich) have established a Center for Integrative Human Physiology with a special research program to support postgraduate education. Funding by the Taiwan government (Ministry of Science and Technology, MoST) promotes grant writing for Physiology and Anatomy majors and advance academic and research performance and communication by physiologists of the Chinese Physiological Society in Taipei.

Clearly, there is a need to leverage funds from either government or philanthropic organizations to support research activities of a large number of global societies dedicated to strengthening physiology as a discipline. Research funds become the first casualty in times of economic stress. Physiologists must play a central role in reversing this trend with a solidly evidenced-base case that solid research in basic and applied science benefits the health, well-being, and wealth accorded to countries.

Recommendations

Societies should explore new means to leverage funding from government and private sources, to aid the development of new initiatives designed to strengthen the discipline.

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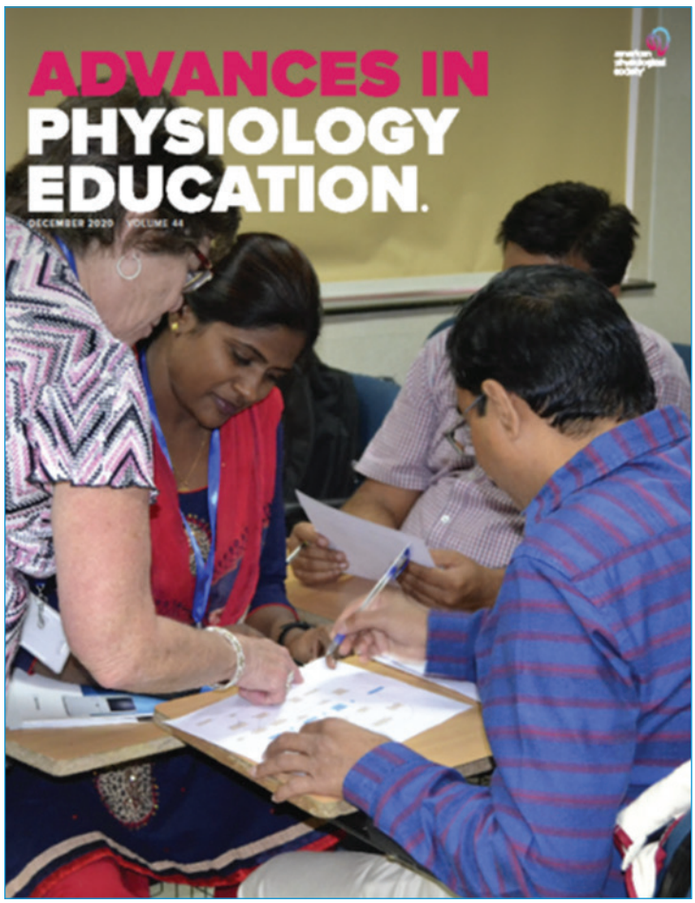
A public engagement display on 'Can exercise make your bones stronger?' at the 2019 President's Lecture where participants could walk, jump and run to find out what this does to our bones and how this changes when we're in space. Jessica Piasecki, Reece Scott and Ian Varley. Nottingham Trent University, UK. Courtesy: The Physiological Society.



On the cover: Dr. Susan M. Barman (left) interacts with a group of physiology educators [clockwise: Dr. Vandana Dudhamal, Dr. Ishaan Kalavatia (partially hidden), and Dr. Srikant Sethe] from several institutions in Northern India as they complete a concept map that integrates the sequence of neural and hormonal events related to the regulation of blood pressure. This active learning strategy was one module of the Case Base Learning session held at All India Institute of Medical Sciences (AIIMS), Jodhpur, Rajasthan (November 3–5, 2018) as part of a series of International Union of Physiological Sciences (IUPS) workshops on Physiology Education Techniques. The goal of the workshops was to introduce young faculty members across India to active learning techniques of problem-based learning, case-based learning, and flipped classroom as ways to best impart to their students the complexities and appreciation of physiological sciences in basic and applied fields. Photograph credit: Debabrata Ghosh. (See Chandran et al. IUPS Physiology Education Workshop series in India: organizational mechanics, outcomes, and lessons. Adv Physiol Edu 44: 525–539, 2020; doi:10.1152/advan.00014.2020).

Technical Expertise and Experimental Models

The need for sharing of technical expertise is paramount in today's world where we need rapid progress in creating new strategies/vaccines/treatments for harnessing the global health care needs. Besides data-sharing as a powerful feature of global health research with regions which bear the heaviest burden of disease, there is a need for collaborative efforts in sharing "hands on" technical skills and equipment as avenues for developing technical expertise. Joseph Travis, President of the American Institute of Biological Sciences, opened its annual meeting in December 2017 with a grand challenge: "*The scientific community must revisit its shared priorities and review existing strategies for decision-makers, from the public to the halls of Congress. Together, the biological sciences community must address three goals: (1) To increase public appreciation for and confidence in science, (2) to facilitate the timely use of science for informing policy decisions, and (3) to ensure the health of the scientific enterprise into the future.*" (Gropp, *BioScience* 68: 643–648. © 2018 American Institute of Biological Sciences). Yet we find that at the time of facing a global crisis with the novel COVID-19 pandemic, Stanley Litow, Professor at Duke University, USA) commented "*Before the coronavirus changed everything, it was common to refer to America's skills crises as the equivalent of a ticking time bomb. A robust but changing economy had exposed the fact that too few Americans had the education and skills needed to fully participate in a workforce that required higher and higher levels of education and skills.*" (https://www.barrons.com/articles/america-already-had-a-skills-crisis-then-the-coronavirus-hit-51584990970). We cannot even begin to imagine the plight of the much smaller and more technically challenged nations in their fight against the novel Coronavirus pandemic that has currently overtaken all countries in the globe. We owe to all physiologists across the globe our endeavor towards creating networks, platforms, and opportunities in the development of technical skills for learning and research in physiological sciences.

Animal models have been widely used to address a variety of scientific questions from basic science to development and assessment of novel vaccines, or therapies. In 1988, the *Use of Laboratory Animals in Biomedical and Behavioral Research*, the National Research Council (US), and the Institute of Medicine (US) Committee concluded that "*Animal experimentation has enormously benefited humans, as well as animals, in the past and will continue to be necessary for clinical and basic research in the future. Indeed, there is no reason to believe that animal experimentation will be less productive in the future… The committee affirms the principle of humane care of all animals, including those used in research.*" (National Academies Press (US); 1988; PMID: 25032311 NBK218273 DOI: 10.17226/1098).

In 2006, the Academy of Medical Sciences, Medical Research Council, Royal Society, and Wellcome Trust sponsored the Weatherall Report on the use of nonhuman primates in biomedical research (royalsociety.org/topicspolicy/publications/2006/weatherall-report/). They forwarded 16 recommendations that included "*a strong scientific case for the carefully regulated use of non-human primates where there are no other means to address clearly defined questions of particular biological or medical importance*" (Recommendation 1) and "*the fields of research considered in this study, namely, communicable disease, neuroscience and reproductive biology, there is a strong scientific case for maintaining the use of non-human primates in some aspects of this work, at least for the immediate future*" (Recommendation 2).

In 2017 a group of researchers based at the Oregon National Primate Research Center in USA examined the critical role played by nonhuman primates in medical research and reported that the rhesus macaque is a reliable animal model to study the impact of Zika virus that was at that time an emerging global threat (Friedman *et al. Pathogens and Immunity* 2: 352-365 2017; doi:10.20411/pi.v2i3.186). By the end of June 2020, the World Health Organization documented 10 million cases and 500,000 deaths, 6 months after the COVID19 outbreak was first recorded in Wuhan, China. Scientists working in various laboratories across the globe

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were already on fast track mode to develop safe and effective vaccines to combat the COVID19 threat. Various species of nonhuman primates have been used as animal models in the pre-clinical trials of such vaccines (Sharpe HR, Gilbride C, Allen E, Belij-Rammerstorfer S, Bissett C, Ewer K. Lamb T. The early landscape of coronavirus disease 2019 vaccine development in the UK and rest of the world. Immunology 2020;160:223-32. doi: 10.1111/imm.13222). Gao and colleagues report, "*The safety and efficacy are essential for vaccine development at both stages of preclinical studies and clinical trials. Although it's still too early to define the best animal model for studying SARS-CoV-2 infections, rhesus macaques that mimic COVID-19-like symptoms after SARS-CoV-2 infection appear promising animal models for studying the disease.*" (Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, *et al.* Development of an inactivated vaccine candidate for SARS-COV-2. Science 2020;369:77-81; doi:10.1126/science.abc1932).

Based on responses received from thirty physiological societies around the globe, we provide here an overview of issues that relate to the development of technical skills in physiological sciences currently interfaced across nations and our future targets.

The Physiological Society offers travel grants for teachers to collaborate or attend conferences to develop their technical skills as an activity of the Society independent of IUPS. The American Physiological Society is respected as an organization that promotes and supports physiology throughout the world and suggests that the role of the Society would be to support this effort of the IUPS, although funding becomes the limiting factor. The National Institute for Physiological Sciences (Japan) holds a physiological science technical training course in which more than 100 trainees take part every year. It also organizes an international internship. Other institutions also organize training opportunities.

In Russia a general tendency now of the Russian Academy of Sciences, Russian Physiological Society is not in the presentation of data at meetings and conferences (since data can be easily accessed through Internet). Rather, physiologists organize inter-disciplinary conferences of mathematicians, physicists, biologists, and physiologists devoted to singling out and discussing unsolved problems in physiology topics include integrative functioning of the organism, cognitive studies, and mechanisms of consciousness aimed to overcoming scientific problems. A joint forum by the Russian Physiological Society, the Russian Association of Artificial Intelligence, the Interregional Association of Cognitive Research, and the Association of Neuroinformatics held an International Conference on Cognitive Sciences (9th : 2020 : Moscow, Russia) in virtual format on October 10-16, 2020. Practically all planned talks were delivered. A book of selected conference papers is published by Springer: https://searchworks.stanford.edu/view/13856190.

The Life Science Switzerland (LS2)-Physiology section reports the need for developing PhD programs that enforce collaboration on education and research focusing on integrative physiology. The Turkish Association of Physiological Sciences requests that the IUPS arrange courses in different countries in collaboration with local societies. The Physiological Society of Nigeria seeks an IUPS global mentorship scheme that could provide opportunities for young physiologists to acquire new technical skills that would benefit research. The Pakistan Physiological Society proposes that IUPS collaborate with their organization to hold Seminars/Workshops/Hands-on Practice/ Refresher courses for researchers in the field of physiology and teachers actively engaged in teaching. Research training in collaboration with IUPS in developed countries would allow scientists to return home with equipment donated by the IUPS or another agency so that science can continue in Pakistan.

The Physiological Society of Southern Africa has proposed to strengthen ties with IUPS to facilitate workshops aimed at improving research and physiology education through exchange of knowledge and resources with countries worldwide. A similar view is shared by the Chinese Physiological Society of Taiwan; they are willing to join the IUPS work force to promote the learning and research in physiological sciences. The Korean Physiological Society proposes that the IUPS program workshops in main meetings designed to help scientists learn certain technical skills. The workshop may encourage the active exchange of the laboratory know-how to facilitate scientific development. This view is also stressed by The Physiological Society of New Zealand who desire the development of workshops that would upskill physiologists in how to handle big data. The Iranian Society of Physiology and Pharmacology considers the importance of conducting workshops for teaching different research skills, increasing international communications, and conducting national and international educational courses. These views of co-organization of workshops with leading experts in the field and the

international exchange of junior and senior teachers/scientists with the involvement of the IUPS experts were shared by many societies including Bangladesh Physiological Society, Brazilian Physiological Society, Bulgarian Physiological Society, Chinese Association for Physiological Sciences, Czech Physiological Society Slovenian Physiological Society, Slovak Physiological Society, and Cuban Society of Physiological Sciences. The Australian Physiological Society is willing to collaborate with the IUPS in this venture aimed at technical skill development in physiological sciences.

The Argentinean Society of Physiology proposes to start a mentorship platform for the Latin American members of the Latin America Society, while the Chilean Society of Physiological Sciences will conduct meetings to promote the concept of collaboration among its experienced members and the next generation with internalization of learning. Physiologists in Spain and Mexico suggest the need for web-based learning and developing teaching material and internet resources for students to exchange new ideas about research methodology, as well as science-based learning methods among IUPS networks of teachers, educators, and researchers. Promoting scholarship of teaching and pedagogical research among physiologists is the suggestion received from the Finnish Physiological Society. The French Physiological Society plans to organize workshops on "*innovative pedagogic methods of teaching*" physiology and seeks financial assistance from the IUPS as this event will attract the audience from France and French-Speaking African countries.

The Physiological Society of India in a collaboration with the South Asian Association of Physiologists organized a Young Scientist Program for young faculty members from Nepal, Sri Lanka, Bangladesh, and India to promote their laboratory skills in physiology and ergonomics, sports physiology, molecular biology, and biotechnology. The event in Kolkata, India included faculty mentors drawn from the Bose Institute, the Indian Institute of Chemical Biology, the National Institute of Cholera and Enteric Diseases, and the University of Calcutta.

Since 2015, the All India Institute of Medical Sciences has held an annual workshop on Techniques in Physiological Sciences (TIPS). Young faculty members from the SAARC nations (Afghanistan, Bangladesh, Bhutan, India, the Maldives, Nepal, Pakistan, and Sri Lanka) attend for the dissemination of technical skills. An Advanced TIPS workshop was conducted in 2019 to provide hands-on expertise in several TIPS that included the following:

- Assessment of Cardiovascular Autonomic Functions through a standard battery of tests (Ewing's Battery) and study of heart rate variability
- Recording of Bereitschafts Potentials that represent cortical activity during motor planning and execution based on amplitude, slope, and onset of these potentials that help to understand the physiology of movement and developmental and neurological, psychiatric, and degenerative disorders that affect movement
- Cognitive Function Testing with test administration, scoring, analysis, and interpretation toward understanding brain functions and behavioral phenomena to aid in the diagnosis/prognosis of patients
- Quantitative EEG (QEEG) to provide "hands on" training on QEEG acquisition, pre-processing and analyses with the aim of increasing an awareness of how this tool is employed in the assessment of brain functions in health and disease
- Pain assessment in humans and animals through analyses and interpretation of recorded data of phasic/tonic pain to gain an insight into spinal and cortical mechanisms of pain modulation
- Whole night polysomnography as "hands-on" exposure on artifact free recording and monitoring of whole night polysomnography in human subjects and scoring of sleep stages, arousals, respiratory, and cardiac events
- Transcranial magnetic stimulation as noninvasive technique to modulate brain functioning through identification, localization, and stimulation of different brain areas to acquire motor evoked potentials and setting the stimulus parameters for delivering transcranial magnetic pulses
- Yoga-based meditation procedures were demonstrated using live performance, lecture, and interactive discussions held by yoga experts and the study of yoga-based meditation on brain functions using EEG and functional near infrared spectroscopy
- Assessment of vascular function with "hands-on" exposure to techniques for assessment of arterial stiffness (Pulse wave velocity, Augmentation index, and Ankle-Brachial index) and endothelial function (flow-mediated dilatation) and cerebral autoregulation (using Transcranial Doppler)
- Pulmonary function testing by spirometry and impulse oscillometry and analyses of collected data
- Methodological Basis of Expressional Microarray to

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Functional Genomics through working knowledge about the procedural details of running expression microarray, data mining and statistics for exploratory and differential analyses followed using bioinformatics web portal and deciphering functional genomics of the analyzed input data.

The figure in the next page provides a glimpse of a few of these techniques in operation at the TIPS-2019 Workshop for sharing of "hands on" technical expertise in physiological sciences with young physiologists from SAARC nations in AIIMS, New Delhi, India.

Researcher at work. Courtesy: the American Physiological Society.

Recommendations

Networks and working groups should be created, domestically and internationally, by IUPS and member societies to facilitate the exchange of knowledge and best practice in teaching and research.

Physiological Sciences: Translational and Regenerative Medicine

The concept of regenerative medicine is an emerging multidisciplinary field to revolutionize the way to improve the health and quality of life by restoring, maintaining, and enhancing tissue and functions of organs. It is a collective field that uses technologies and seeks to develop functional cell, tissue, and organ substitutes to repair, replace, and enhance biological function that has been lost due to congenital abnormalities, injury, disease, and aging.

Scientists who have worked with isolated cells share an immense fascination and joy while observing how isolated living cells grow and interact with matrix molecules to generate basement membrane, assume cell polarity, form gland-like structures, and undertake functional differentiation in the near-physiological environment to form tissue-like structures *in vitro*. The foundation of our current understanding of cell biology and regenerative medicine emanated from the critical studies initiated in the 1960s and 1970s with an attempt to mimic *in vitro* the *in vivo* conditions. This included attempts to examine how isolated cells behave under conditions of optimal oxygenation and hydration, adapting to the given niche to proliferate, migrate, and invade in the presence of a homogenous or heterogenous population of cells and extracellular matrices. Thus, we find evidence of skin-equivalent tissue developed *in vitro* from the laboratory of Eugene Bell and his colleagues in 1981 (*Science* 211:1052-1054, 1981). The advent of embryonic stem cells from two laboratories (Evans MJ, Kaufman MH. *Nature* 292:154-156, 1981; Martin GR. Proc Natl Acad Sci USA 1981;78:7634-8) and the development of human embryonic stem cell (hESCs) lines *in vitro* from human blastocyst (Thomson JA, *et al. Science* 1988;282:1145-7) ushered a new era in regenerative and translational medicine. In the presence of appropriate differentiating factors and culture conditions researchers predict that hESCs can be used to create specialized cells to treat a variety of diseases that include Parkinson disease, Alzheimer disease, cancer, spinal cord injury and juvenile-onset diabetes and also be effective models in the development and testing of new drugs. Shinya Yamanaka (Takahashi K, Yamanaka S. *Cell* 2006;126:663-76) engineered adult mouse cells to return to an embryonic-like state by adding four transcription factors, the availability of these induced pluripotent stem cells help to circumvent some of the ethical issues that surround the use of hESCs that are generated from cells derived from "spare embryos."

Tremendous advances have now been made in the interdisciplinary fields of biological sciences and engineering to develop tissues that aim to restore, maintain, and enhance tissue function and to provide a solution to organ failures. Tissue engineering is an "interdisciplinary field that applies the principles and methods of engineering and the life sciences toward the development of biological substitutes that can restore, maintain, or improve tissue function." (Fuchs *et al.*, *Ann Thorac Surg* 2001;72:577-91). Matching tissue engineering technologies with biological and medical needs requires the integration of various scientific disciplines, such as physics, cell biology, and developmental biology.

Miranda Grounds (*Clin Exp Pharmacol Physiol* 2018;45:390-400) offers an excellent overview of this complex area of bioengineering and translational medicine that includes the advances that have been made in the areas of cell culture models, scaffolds, the use of nanotechnology and computer modeling, the use of *in vivo* animal models, and the success as well as the challenges ahead to increase transplantation success in the clinical scenario.

The nature of the scientific question under study in most cases helps to decide the selection of the most appropriate investigative models and endpoints. For example, the similarities in anatomy and physiology of large animals such as dogs, pigs, and sheep to the human heart enable translational research in devising imaging tools and to develop therapeutic devices such as catheters, stents, and valves. There are some limitations in using large animals as scientists need to keep full transparency and implement the 3Rs principle (reduce, replace, and refine) in their experimental set-up as they produce reliable and high-quality data in highly competitive

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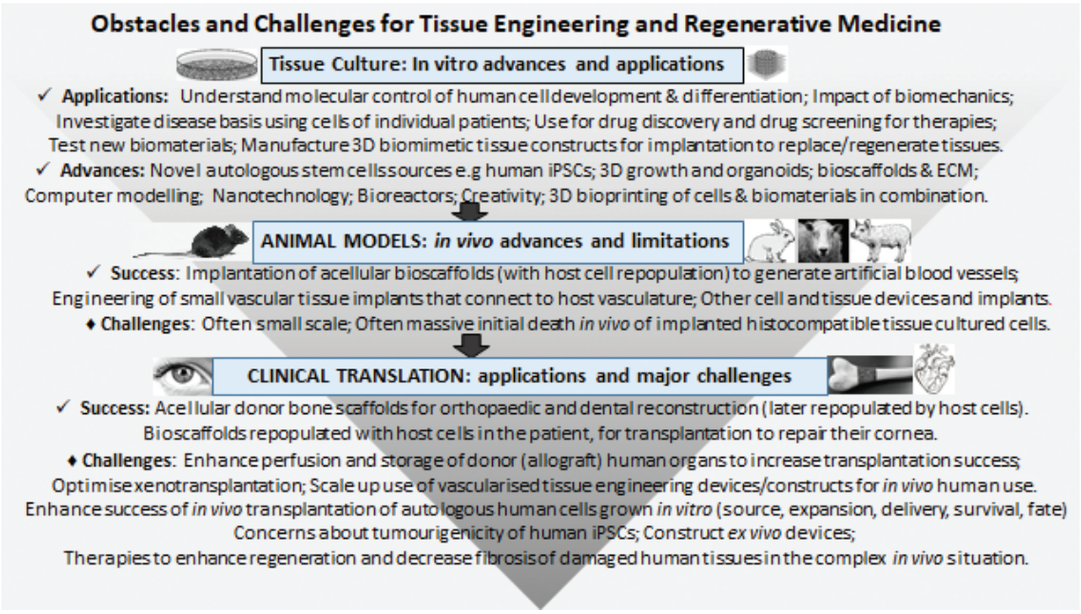

Summary of recent activities in Tissue Engineering and Regenerative Medicine that expand the activities in tissue culture combined with progress for *in vivo* applications using animal models (e.g., rodents and larger species), and the major problems and challenges in clinical translation to humans. With the kind permission of M. D. Grounds, School of Human Sciences, the University of Western Australia, Perth, WA, Australia (*Clin Exp Pharmacol Physiol* 45:390–400, 2018).

fields such as cardiology (Cesarovic N, Lipiski M, Falk V, Emmert MY. Clinical relevance and translational limitations of animal models in cardiovascular medicine *Europ Heart J* 2020;41:200-3; doi.org/10.1093/eurheartj/ehz933; Bert B, Dorendahl A, Leich N, Vietze J, Steinfath M, Chmielewska J, *et al.* Rethinking 3R strategies: Digging deeper into *AnimalTestInfo* promotes transparency in *in vivo* biomedical research. *PLoS Biol* 2017;15:e2003217; doi.org/10.1371/journal.pbio.2003217).

In the process of generating a Status Report on Physiological Sciences across the globe, the BGA of the IUPS had raised a query about the current road map toward translational and regenerative medicine and the role played by physiologists. We have received responses from twenty-six societies. In Table 10, we show how physiologists are involved in making progress in these advanced areas of research and clinical translation.

Tissue engineering is an interdisciplinary field that involves cell biology, materials science, reactor engineering, and clinical research with the goal of creating new tissues and organs (Lavik, E. Langer R. Tissue engineering: current state and perspectives. *Appl Microbiol Biotechnol* 2004;65:1-8. doi: 10.1007/s00253-004-1580-z.). Clearly, the knowledge of cell biology and developmental biology that allows the growth of cells and tissues *in vitro* as organoids and organs ready for transplantation would require matching integration with disciplines such as mathematics, physics, and tissue engineering technologies to accomplish the goal of regenerative medicine. The recognition of physiology education by engineering institutions and inter-alia active collaboration between physiologists and biomedical engineers is the need of the hour. The American Physiological Society remarked that institutions such as Massachusetts Institute of Technology and others are engaged in activities to collaborate with health centers to provide specific funding for joint

Training clinical skills - and physiology. Medical students taking patient's pulse and auscultating the heart and abdomen. Simulation training is centred in the Skills Lab of the Medical Faculty of University of Helsinki, where facilities can be flexibly transformed to resemble authentic multi-professional working environment in hospital wards or health centres. Physiologists use a variety of simulators to illustrate the functions of the human body. The staff of Skills Lab consists of health care professionals and technicians. Students have the opportunity to reserve facilities for independent practice, or under the guidance of tutorial nurses and peer tutors. Text by Liisa M Peltonen. Photo by Teemu Masalin. Courtesy: University of Helsinki and Finnish Physiological Society.

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Table 10: Applications of basic research to clinical medicine.

Member organization	Regional involvement in advancing research in translational and regenerative medicine and development of biomedical devices
American Physiological Society	• The major federal funding agency NIH links research to translational outcomes through the 20th Century Cures Act and via a new division of translational science. This involves increased regenerative medicine initiatives. In addition, individual, specialized charities, and organizations sponsor translational research into specific diseases
Argentinean Society of Physiology	• Regenerative medicine is well developed in some institutions. Translational medicine is not so well developed but it is encouraged by the National Research Council, in Argentina
Association of Physiologists and Pharmacologists of India	• The School of International Biodesign, New Delhi and the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum have taken steps to translate basic research into biomedical devices for clinical application
Bulgarian Society for Physiological Sciences	• Centers of excellence and centers of high achievements in tissue engineering and gene therapy development
Canadian Physiological Society	• Multi-disciplinary research funding opportunities are linked by multiple national funding agencies to combine expertise in life sciences with engineering (e.g., CHRP - Collaborative Health Research Program). Mitacs is one funding agency that is designed specifically for collaborative Academic-Industry projects (including biotech/life sciences) with the involvement of trainees
Chilean Society of Physiological Sciences	• Projects in translational medicine are funded by the, National Commission for Scientific and Technological Research (CONICYT), Fund for Research Centers of Excellence (FONDAP)
Chinese Association for Physiological Sciences	• Conducting summer training courses on scientific paper and fund writing, classroom teaching, and experimental teaching of physiology
Chinese Physiological Society in Taipei	• Government launched national programs to facilitate the development of university-operated Incubator, university-affiliated biotech companies, and regional science parks to host start-ups that transfer novel techniques/research outcomes from near-by universities
Cuban Society of Physiological Sciences	• Cuba is developing translational medicine, regenerative medicine with bone marrow stem cells. There is development of medical devices and vaccines based on research in basic sciences with applications to the Cuban people and to other countries
Czech Physiological Society	• There are several research institutes and biomedicine enterprises aimed at application of this research into business. This field especially involves the development of drugs, development of new biomedicine devices and partly also regenerative medicine
Finnish Physiological Society	• Start-up companies focus on biomaterials (in dentistry and orthopedics), drug development and diagnostics. The Euro-Bioimaging Centre, ERIC is in Turku Science Park (www.bioimaging.fi)

(*Contd...*)

Table 10: (*Continued*).

Member organization	Regional involvement in advancing research in translational and regenerative medicine and development of biomedical devices
French Physiological Society	• There is encouragement in the setting-up of spin-off projects in these fields
Hungarian Physiological Society	• Semmelweis University in Budapest, University of Szeged, and University of Pecs each have department of translational medicine. These departments are based on former departments of pathophysiology with new curricula aimed to focus on making a bridge between basic science and clinical practice. In Szeged there is a 3D printing center for advanced research in regenerative medicine
Iranian Society of Physiology and Pharmacology	• Advanced research using Next Generation Sequencing (NGS) techniques for cancer diagnosis and therapy and performing advanced preclinical studies for cell therapy in different diseases
Israel Society of Physiology and Pharmacology	• Israeli science is deeply engaged in translational and regenerative medicine
Korean Physiological Society	• Research in translational medicine and regenerative medicine is ongoing. The society encourages collaborative work between departments of engineering and physiology for development of medical devices
Life Science Switzerland (LS2)-Physiology	•University research programs on personalized medicine (e.g., Hochschulmedizin Zurich), Technical Center for Studying Human Health (e.g., Swiss integrative Center for Human Health (SICHH) at University of Fribourg, Switzerland) have been developed to encourage research in these fields
Physiological Society of India	• Government organizations like Central Drug Research Institute, Central Toxicological Laboratory both in Lucknow, AYUSH (Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy) and university faculty members are involved in research in many aspects of translational medicine, regenerative medicine, and in the development of medical devices. This is substantiated from various drugs developed and biomedical devices, patents filed, and clinical trials undertaken. Traditional plant products having ethnopharmacological importance to be reviewed for their potential in the prevention of different diseases with use of modern technologies in the current genomic era
Physiological Society of Japan	• Activities toward regenerative medicine in Japan are prominent with a strong support by the government, after the establishment of iPS cell by Nobel Laureate, Prof Yamanaka. Approaches using Multilineage-differentiating stress-enduring (Muse) cells, "Endogenous" pluripotent stem cells, also attract high attention.
Physiological Society of New Zealand	• Through government funding of Centers of Research Excellence is investment into the development of medical devices. This has led to the spin out of startup medical device companies that are employing physiology and bioengineering graduates

(*Contd...*)

Table 10: (*Continued*).

Member organization	Regional involvement in advancing research in translational and regenerative medicine and development of biomedical devices
Physiological Society of Southern Africa	• Regionally there is a move for the validation of ethnomedicinal plants and phytochemicals for clinical use.
Russian Academy of Sciences, Russian Physiological Society	• The members of this society are involved in brain-computer interface, molecular mechanisms of stress, molecular mechanisms of learning, memory, cognitive abilities, and neurogenetics
Slovak Physiological Society	• Centers for excellence in translational research having open calls for projects promoting research in above-mentioned areas. Support for projects based on cooperation between academia and industry
Slovenian Physiological Society	• Autologous cell-based anti-cancer vaccines and cell-based immunotherapy. Electrochemotherapy to target surface tumors. Cartilage repair with autologous chondrocytes. Development of small molecules to rescue cholinergic and noradrenergic decline in neurodegeneration
The Physiological Society	• The society is holding a meeting on Regeneration in 2021
"Turkish Association of Physiological Sciences"	• Neuroscience has evolved into translational medicine, regenerative medicine, and development of medical devices in Turkey rather than physiology

projects between engineers, fundamental scientists, and clinicians. At the Zilina University in Slovakia, the program of Biomedical Engineering has physiologists from medical faculties teaching Physiology; and vice versa – many engineers are in PhD and postdoc positions at Universities and Academy. There is strong interaction between physiologists and engineers in New Zealand through collaboration with the Auckland Bioengineering Institute and the MedTech Centre of Research Excellence. Iranian physiologists have good interactions between their Medical, Biological, and Engineering Faculties in designing and making new instruments software and advanced data analysis. Physiologists have developed new instruments for experimental and clinical applications. In Japan, there is good collaboration between physiologists and biomedical engineers in sorting out the effectiveness and usage of biomedical devices.

The scenario in India is also supportive with physiologists from the basic sciences being engaged as scientists/faculties in several renowned institutions of engineering to teach in courses for biomedical engineering, biotechnology, microbiology, and marine engineering, design and ergonomics at Institute for Occupational Health (Ahmedabad and Kolkata), National Institute of Technology, Indian Institute of Engineering Science and Technology (Kolkata), and Indian Institute of Technology (Kharagpur, Mumbai, Delhi, Kanpur, Guwahati and others). In Hungary, several education programs are being operated in Budapest for biomedical engineering for more than 30 years. The Czech Physiological Society reports that every technical university in Czechoslovakia offers education in the field of biomedical engineering and includes lectures from human physiology, biology, and related topics with reasonable research being performed in the field of bioengineering.

In several countries including Chile, China, Cuba, France, Nigeria, Russia, Slovenia, South Africa, Switzerland, Taiwan, and Turkey, there is close collaboration in teaching and research between physiologists and biomedical engineers. In Bangladesh, physiologists are hired to teach in engineering colleges. In Nepal, physiologists realize the need for integration of physiology in programs such as biomedical engineering, but these are not being executed till date. The Canadian Physiological Society requests that the IUPS provide leads and opportunities to collaborate with other researchers in the fields of translational and regenerative medicine to help strengthen profiles and representation on a global level.

Combining theory to practice. A small group of students have just practiced in pairs the observation, palpation and percussion of the abdomen, while repeating the surface anatomy of the abdomen. Now they are using their own phones to access a patient case related to intestinal function. The mannequin patient lies tight on the bed waiting for inspection. After familiarization and short discussion, students listen to the intestinal sounds associated with the case and together with the instructors, reflect on their findings and relate them to intestinal motility and general gastrointestinal physiology. The instructors are young doctors who are trained not only in physiology but also in pedagogy. Text by Liisa M Peltonen. Photo by Teemu Masalin. Courtesy: University of Helsinki and Finnish Physiological Society.

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Something old, something new. When money is sparse, even outdated equipment from hospital skips may be most helpful after appropriate tuning. Text by Liisa M Peltonen. Photo by Teemu Masalin. Courtesy: University of Helsinki and Finnish Physiological Society.

Recommendations

Networks and working groups should be created, domestically and internationally, by IUPS and member societies to facilitate the exchange of knowledge and best practice in teaching and research.

IUPS should oversee a new Global Mentorship Building Platform to facilitate Mentor/Mentee relationships among physiologists at various career stages, and in academic and clinical settings, to promote dialog and aid career development.

Physiome Project and Virtual Physiological Human: Applications in the Health Care Needs of Society

The concept of the Physiome Project was first presented in a report from the Commission on Bioengineering in Physiology to the International Union of Physiological Sciences (IUPS) Council at the 32nd World Congress in Glasgow in 1993. Physiome derives from "physio" (life) + "ome" (as a whole) and is intended to provide a quantitative description of physiological dynamics and functional behavior of the intact organism. James Bassingthwaighte described "*the physiome as the quantitative description of the functioning organism in normal and pathophysiological states. The human physiome can be regarded as the virtual human. It is built upon the morphome, the quantitative description of anatomical structure, chemical and biochemical composition, and material properties of an intact organism, including its genome, proteome, cell, tissue, and organ structures up to those of the whole intact being. The Physiome Project is a multicentric integrated program to design, develop, implement, test and document, archive and disseminate quantitative information, and integrative models of the functional behavior of molecules, organelles, cells, tissues, organs, and intact organisms from bacteria to man*" (Bassingthwaighte, 2000). As the Chair of the IUPS Commission on Bioengineering in Physiology, he had chaired a satellite workshop "*On designing the Physiome Project*" in Petrodvoretz, Russia, following the 33rd World Congress in St. Petersburg in 1997. It then became a major focus of the IUPS in the millennium, with a synthesis on the Physiome Project at the 34th World Congress of IUPS in Christchurch, New Zealand, in 2001. Denis Noble visualized that "*Physiology should be providing the synthesis to match the reductionist analysis of molecular biology by integrating all the molecular information into an understanding of physiological function ["putting Humpty Dumpty together again"]*" (Biochem. Soc. Trans. 2003;31:156-58).

In 2005, a small group of researchers discussed how the vision of computational physiology promoted by the Physiome Project could be translated to clinical practice. They formally proposed the term *Virtual Physiological Human*. In *A vision and strategy for the virtual physiological human in 2010 and beyond*, Hunter P, Coveney PV, de Bono B, Diaz V, Fenner J, Frangi AF, Harris P, Hose R, Kohl P, Lawford P, McCormack K, Mendes M, Omholt S, Quarteroni A, Skår J, Tegner J, Randall Thomas S, Tollis I, Tsamardinos I, van Beek JH, Viceconti M. (Phil. Trans. R. Soc. A, 368, 2595–2614, 2010) called for a need "*...to implement biomedical research outputs into clinical practice and healthcare industries, we need to integrate data, information, knowledge and wisdom. We need to integrate data of the same patient stored in different hospitals in different member states or in clinical research databases; we need to integrate the information related to various parts and processes of the human body into a systemic understanding of pathophysiology; we need to integrate the knowledge digitally captured into metadata, ontologies and models to respond to the combinatorial explosion of cognitive complexity that integrative research is producing; and we need to integrate the wisdom produced in the research laboratories and in clinical practice, which will be formalized in guidelines, standards and protocols and used to promote translation of basic science and integrative models into healthcare benefits.*"

The first International Symposium on Systems Biology in Physiology/Medicine held in New Delhi, India in 2011 at the All India Institute of Medical Sciences was chaired by D. Ghosh as a part of the 57th Annual Meeting of the Association of Physiologists and Pharmacologists of India. Excerpts of the meeting can be accessed at *Indian J Physiol Pharmacol*, Vol. 55, No. 5, Supplement, 2011; www.ijpp.com. The aim of this meeting was to encourage physiologists in India to ponder the definition of Systems Biology and how it may be promoted toward translational medicine and health care. In his plenary lecture, Denis Noble defined "*systems biology as a theory of biological relativity in which the first principle is that there is no privileged level of causality* (Noble D. Genes and Causation. *Philosophical Transactions of the Royal Society*

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A 2008;366:3001-15; Noble D. A Theory of Biological Relativity: no privileged level of causation. *Journal of the Royal Society Interface Focus 2*, doi: 10.1098/rsfs.2011.0067, 2012). Charles Auffray opined, "*We conjecture that biological systems self-organize because they operate as a conjunction between the relatively variable part of a stable organization and the relatively stable part of a chaotic network of fluctuations, and in a space with a changing number of dimensions: biological space-time.*" In his lecture on "The Physiome Projects: Modeling toward Human Health," Jim Bassingthwaighte cautioned physiologists, "*The ease of understanding models however diminishes as the complexity increases, necessitating better interfaces and broader tutorials and operational instructions. Modular construction favors identifiability of elements and the understanding of components. With new methods one can automate the reconstruction of large models when components are modified, or new ones added.*" A typical systems biology approach was further explained by Gérard Siest when he concluded "*integrating genetic data, transcriptomic and protein ones, is the strategy for developing pharmacogenomics for personalizing the treatment and avoiding side effects and drugs interactions.*"

In 2016, a major investment in research in the virtual physiological human (VPH) was being defined as a "*methodological and technological framework that, once established, will enable collaborative investigation of the human body as a single complex system.*" This was triggered through seeding of the EuroPhysiome: A Roadmap to the VPH (STEP, http://www.vph-institute.org/upload/step-vph-roadmap-printed-3_5192459539f3c.pdf).

Three guiding principles of the Physiome Project were developed as discussion points with physiological societies across the globe to relate to the current status of physiological sciences:

i. How can physiologists develop and encourage model (physiome)-driven physiological research?
ii. Identify future research directions of physiological sciences in the era of data driven science including artificial intelligence (AI)
iii. What is needed to be done in physiological science in the era of individualized health care?

The American Physiological Society explains physiology as being the study of life-processes, several of which (e.g., blood pressure, heart rate, respiration, blood chemistry, sleep patterns, and more) can be recorded and monitored on a continual basis through wearable technology as modes to encourage model (physiome-driven) physiological research. Many disease conditions can be picked up at an early stage by continual monitoring, rather than a once per year snapshot of an individual's health parameters. The American Physiological Society recommends:

* Use of large electronic medical records to identify datasets in establishing relationship of physiological parameters to diseases
* Collect data of several critical physiological parameters based on continual health monitoring rather than data collected at irregular intervals
* Use AI to analyze physiological recordings and then identify potential health issues, based on searches of large databases
* Use of AI to predict disease susceptibility, drug reactions/tolerance, and outcomes of interventions
* To link genetic and environmental factors, and cellular pathway data, to health status and individualize treatment strategies as effective modes to drive physiological science in individualized health-care programs.

The Argentinean Physiological Society reported that the Physiome Project is being developed in Argentina by scientists at the Favaloro Foundation who have been collaborating since 2013 in areas of modeling by combining experimental and *in silico* data with papers being published in prestigious journals. To outreach and follow the VPH Initiative's main goals to develop patient-specific computer models for personalized healthcare and simulate disease-related processes, physiologists should make an effort to understand this initiative and be supported by collaborative programs in each country.

Physiologists of the Chilean Society of Physiological Sciences are engaged in translational physiology, making applications to support the concepts of physiology and pathophysiology together. Physiologists in Chile consider that massive data collection should be conducted as part of understanding the physiology behind diseases to ensure patient-specific

research, particularly in areas such as obesity and associated diseases.

The Canadian Physiological Society considers that physiologists should now focus on underlying mechanisms of disease and regulation of physiological parameters. New approaches in data science and genetics will help to generate significant opportunities to understand previously unrecognized systems in all realms of physiology. Efforts are underway in some Canadian provinces to develop large health record databases. Future developments will enable linking of patient physiological data with genetic data. The establishment of these large and complex datasets in Canada and elsewhere has the potential to be incorporated with AI to relate genetic and physiological features. Individualized health care will reveal predictive traits in terms of patient responses to treatment. Physiological sciences should focus on the underlying mechanisms that govern different patient responses to treatments. Physiologists should also aim to identify "high-yield" scenarios where there are certain cohorts of patients that respond extremely well (or extremely poorly) to certain treatments to leverage modern approaches (genetics, biomarkers, and metabolic profiles) to understand underlying mechanisms.

Physiologists from the Czech Physiological Society are of the opinion that there should be an improvement in the general awareness of applicability of mathematical models by giving examples from health practice while teaching physiology. Semi-automated recognition of imaging and other physiological data and physiology-based simulators should be made available to medical students and trainee doctors. Solid basic research and recognition of subject individually even in basic science will promote development of individualized healthcare with emphasis placed on basic physiological research, including gender specific data collection.

Advancement of the Physiome Project by scientists of the Finnish Physiological Society includes a multi-disciplinary collaboration by engineers, programmers, and data analysts and collaboration with companies selling and developing devices measuring physiological indices. Together with analysis of big data collected from the consumers, using wearable devices (in collaboration with companies) and exploitation of such data collected at home by patients using wearable devices and measurements made at home will advance human health.

Physiome is at the heart of interrelationships with gene-therapy and bio-medical disciplines like metabolomics and genomics. The French Physiological Society stated that an in-depth knowledge of physiology will help inform the design of personalized medicine and nutritional, immunophysiological, and neuro-immuno-endocrinologic studies and lead to more translational research.

The Hungarian Physiological Society opined the need to expand knowledge about the physiology of healthy individuals. In their opinion, there is limited description of age-related changes even in basic physiological parameters. Non-invasive measurements by virtue of telemedicine could help in understanding healthy aging or physiological adaptation to a changing environment (migration etc.). A goal-oriented data collection and evaluation would help in understanding and expanding what is meant by "healthy" in a certain situation. The vast amount of data generated in hospitals may be explored to understand the fine interplay or independency between various types of data collected. Based on AI, new factors can come into focus and some of the traditional lab data can be neglected. While traditionally the morphology of physiological data is analyzed, temporal patterns and changes therein may be considered instead of static data to change our way of thinking about physiology. Due recognition of the history of personal health records from routine physical check-ups beginning at birth and continuing through later ages should be monitored to consider personal prognosis or comparison to the population average. If an unusual change is noted, it can be taken as a warning sign for initiating preventive measures.

The Association of Physiology and Pharmacologists of India considers that Physiome models can be used to identify outcome variables which are likely to respond in a sensitive manner to external or internal perturbations under a given set of conditions. This knowledge may then be used as background evidence for developing measurement techniques/devices for such variables that can be used as physiological markers for monitoring health/disease states. In future research directions, AI can help derive better models of physiological systems. Then, by working back using experimental approaches from the input model variables identified, AI may help to decipher the physiological mechanisms through which they will be affecting the output/outcome variable. In the era of individualized healthcare,

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there is a need to delve deep into understanding the individual specific short-term and long-term longitudinal variabilities and trends in physiological parameters rather than using the population means estimated at limited time points.

Basic science researchers and physiologists of the Physiological Society of India are popularizing the Physiome concept among the undergraduate and postgraduate students and creating interest in this area for the development of signal-based devices for individual health care and its collated feedback for research inputs. In this era of data driven science, AI can help in data storage and prediction based on physiological data such as blood pressure, blood glucose, heart rate, cardiac cycle and output, sleep, and endogenous secretion patterns instantly and ergonomically for the development of best individualized healthcare and personalized medicine. For common but critical diseases, physiological genomic readouts in disease-applicable tissues may replace the effect of genetic, racial, and environmental factors and their interactions that underlie disease development and progression. Physiological genomic readouts in disease-relevant tissues, combined with advanced AI, can be a powerful approach for precision medicine for such diseases. Prediction from epidemiological data of disease occurrence and methods for its cure and prevention in a personalized readout remains an important factor. Physiological data in individuals vary from subject to subject, hence, based on individual epigenetic and molecular data of each subject, precision, and personalized medicine can be developed, that is, delivering the right treatment to the right patient at the right time, usually with a spotlight on a data-centered approach. This practice aided by research data from physiological sciences using biomedical devices will not only ensure specific proper treatment but also lower the chances of ill effects.

Members of the Physiological Society of Japan have developed excellent mathematical models based on experimental data at the level of cardiac myocytes. For further construction at organ and system levels, an integration of data of various layers is required. To meet this aim, it will be critical to share data and appropriate standardization. The goal is to establish quantitative *in silico* human model system, incorporating information of gene/cellular responses, cell-cell network interactions, and inter-organ interactions. It will fully explain the mechanism of homeostasis and show the way to recover from a disturbance. The goal of physiological science in Japan has been, is, and will be to elucidate the functional mechanisms of life. With this understanding, there remains a hesitation to emphasize too much the contribution of personalized medicine. At the same time, there is a need to remain open to translational research for which an interplay between clinical, intermediate, and basic researchers would be effective. The important role of physiological science would be to understand the mechanisms of homeostasis to remain healthy.

The Korean Physiological Society has a physiome research group whose approach lies in the diagnosis of cardiovascular diseases, disease modeling, and drug discovery. Physiologists attempt to develop physiome data-driven physiological indices such as heat capacity, basal metabolic rate, cardiac output, oxygen consumption, and body temperature regulation. In the systems biology field, genomic and proteomic research is being conducted. Machine learning, including deep learning, has been used in biological research including physiology. This system has made good progress in systems biology and network biology. Physiologists see the need to develop methods on how to decipher physiological functions with cheap or available devices to understand how the body maintains its health status. In the era of individualized healthcare, big data and its convergence will open a new field, but the value of individual physiological parameters also needs to be appreciated. In this manner, understanding the microenvironment and metabolism will progress through analysis of complexity, integrated/dissected functional analysis, simulation modeling, and patterning.

The Physiological Society of New Zealand undertook a collaboration with the Auckland Bioengineering Institute which is actively involved in the Physiome initiative under the leadership of Peter Hunter. One high profile area is using GPS Fitness Tracking devices to analyze data related to high performance athletes. In New Zealand, traumatic brain injury and concussion in rugby is an issue. In the era of individualized healthcare, they are aligning physiological data with genomic data.

An approach to the Physiome Project by members of the Physiological Society of Slovakia involves the collection of physiological data and their analyses by appropriate means to have standards for possible variables. For future research

directions of physiological sciences in the era of data driven science, they plan to analyze biological/physiological data by non-standard mathematical methods and develop devices able to detect the disorder before clinical signs occur. They continue their work to understand how the human body is functioning in a holistic approach beside study of genes and molecules.

Members of the Physiological Society of Slovenia see value in the organization of international conferences on "*Physiome*" to strengthen the network of scientists and to open interactions with scientists working in the Physiome project in hopes of attracting new scientists. In accordance with the Physiome project, they support the development of mobile/wearable devices to measure as many physiological data on individual patients in health and disease and *in silico* systems (high-performing computers) to analyze and interpret collected data to understand physiological differences between healthy and diseased individuals. By comparing such compiled data with transcriptome of patients, one can identify underlying mechanisms of altered physiology in individual patients towards developing strategies for identifying underlying molecular mechanisms of physiological disorders in individual patients and to develop strategies to treat patients individually based on the identified patient specific disease characteristics.

A similar approach is envisaged by the Physiological Society of Southern Africa with the use of 24 h ambulatory blood pressure measuring devices and measurement of arterial stiffness over a 24 h period. These wearable devices include ECG monitoring and provide accurate measurements to allow monitoring of patients in their home environment that can be monitored remotely by the researcher/doctor. Furthermore, genotyping will allow for individualized specific medications for such patients.

The Bulgarian Society of Physiological Sciences noted that a multidisciplinary approach that includes a collaboration with mathematicians will aid in exchange of hypotheses and ideas relevant to the physiome project. They also acknowledged that data-driven research needs to be generated to understand how to treat and prevent the onset of disease. Physiologists from Bulgaria participate in national scientific programs such as BioActiveMed to plan and conduct scientific research in the direction of individualized healthcare. Physiologists from the Chinese Association of Physiological Sciences promote the development of physiological instruments with directions to include popular science and continuing physiology education, development of physiological instruments, and Applied Physiology and Translational Neuroscience as well as conducting popular science education of physiology.

The Chinese Physiological Society in Taipei considers it important to ask physiological and mechanism-related questions when serving as peer reviewer for journals and conference presentation. They also propose to conduct preventive and precision medicine in Telehealth Centers. In the era of individualized healthcare, physiological parameters must be set up as a data bank for individuals with next-generation sequencing for intestinal microbiota to explore host-microbe interaction and regulation of human physiology.

The Turkish Association of Physiological Sciences considers the importance of Physiology needs to be recognized as the basis of wellness and illness among lay people and Health Sciences professionals and a demand created for physiome-driven physiological research and services. In Turkey, there are Wellness Centers as subsidiary to Health Centers (primary care), physiologists may be trained especially for exercise and pathophysiological issues, with health applications of mobile devices along with laboratory devices especially for VO_2 max.

The Spanish Physiological Society espouses the need to improve existing physiological models and to train new professionals in such areas. The Iranian Society of Physiology and Pharmacology recommend that physiologists become familiar with and increase the use of computational sciences to obtain/analyze high throughput data and to be familiar with "Omics data." The gathering and banking of such data shall then help in developing new areas of personalized medicine, regenerative medicine, and cell therapy. Physiologists from the Brazilian Physiological Society think that translational research involving physiology and medicine will pave the way towards understanding individual and population-based health care problems. The Nigerian Physiological Society would like to promote research that is focused on how new data may improve life expectancy and the quality of life. The Bangladesh Physiological Society encourages the development of simple wearable devices for monitoring physiological data to enlighten the wearer of his/her health status under normal and adverse health conditions and to promote self-monitoring for a better

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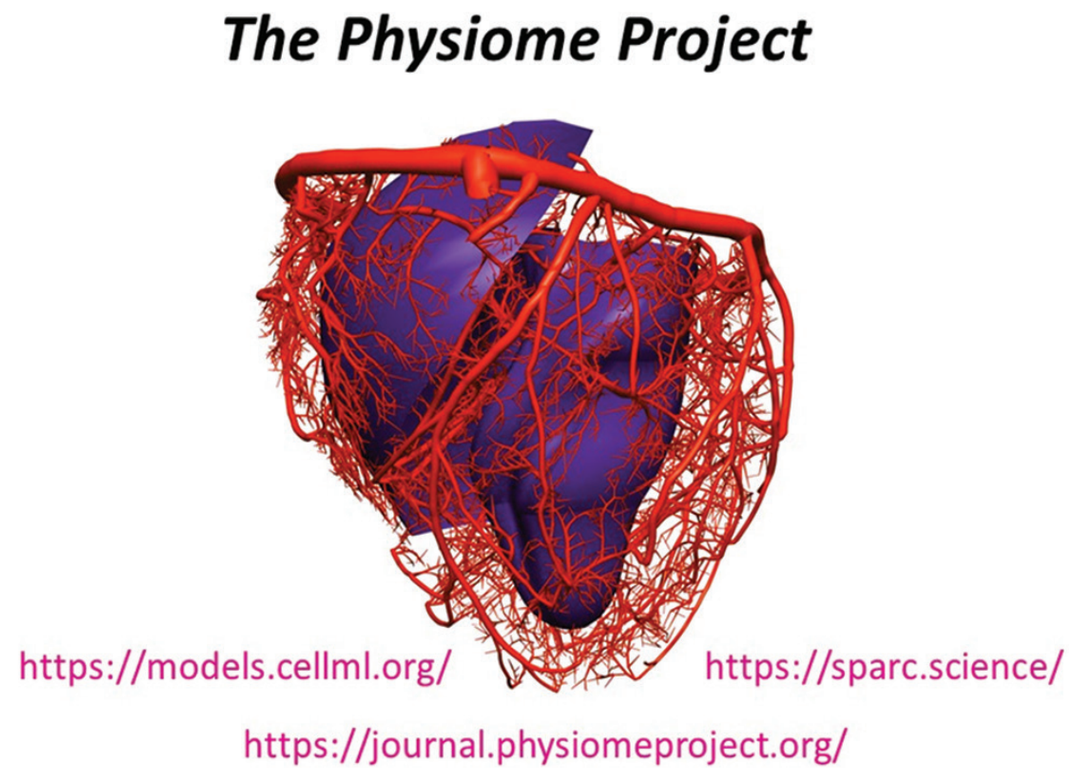

Courtesy: Peter Hunter, University of Auckland, Auckland, New Zealand.

quality of life. Members of the Cuban Society of Physiological Sciences would like to participate in the Physiome project in collaboration with other societies and aim to perform research towards individualized health care.

The Romanian Physiological Society identifies future research directions of physiological sciences in the era of data driven science including AI through the study of the human body as a whole and an in-depth study of correlations among regulating mechanisms of human systems. Emphasis should be placed on molecular pathways involved in maintaining physiological functions and their adjustment to various stimuli. A similar approach is advanced by the Russian Physiological Society; individualized general health care is designated as a priority for physiology and medicine. Physiological expertise and explanations are necessary for any new device; advances in epigenetic regulation of pathologies, molecular mechanisms of organism functioning, cognition, memory, and consciousness are a few of the major directions of research in neurophysiology. A key approach to advance physiological science in the era of individualized healthcare requires a shift to understanding mechanisms of integrative effects of epigenetic regulation of organism functioning.

Physiologists of the Life Science Switzerland (LS2)-Physiology acknowledge the need for animal models in physiome-driven research in physiological sciences. They support the strengthening of close interactions with clinicians and industry to develop non-invasive measurements of physiological parameters in the whole body. The society stressed the need to maintain the use of whole animal physiology for understanding body functions and to develop proper models to validate findings obtained from big data. Addressing the issue of how physiological sciences can advance in the era of individualized healthcare, they highlighted the need to provide and develop animal models and/or experimental models or approaches that allow investigation of "individual metabolic situations" and to conduct necessary tests.

Scientists from different fields including computational and AI sciences are needed to help in the development of models designed to encourage physiome-driven physiological research. This notion was highlighted by The Physiological Society. They reported key progress in some areas linking individual genomics to clinical data and disease susceptibility, targeting pharmacological drugs to individual genotypes, and establishing individual drug metabolism profiles (pharmacodynamics/kinetics) to ensure that target minimum inhibitory concentrations are reached based on whole genome sequence-predicted drug resistance. In addition, growing datasets in genomics, proteomics, peptidomics, and microbiome are now available; and scientists are harvesting published data for drug re-purposing. More globally, comprehensive analyses of large datasets and "open" access to such datasets (health-related data from large cohorts of patients or sets of single-cell or other transcriptomics) are needed for the research community to support hypothesis generation and testing. Besides access to "*raw*" data, researchers need to be trained in the evaluation of these databases; there is a role for AI based analysis in this process. It is necessary to access and identify useful data and cross reference such data for its interrogation with AI. Furthermore, the terminology/cataloging of drugs, ion channels, and cells need to be standardized by physiologists so that there is a uniform nomenclature. It is important to provide more access and funding to facilitate the work of computer scientists having background knowledge of biological sciences.

Recommendations

Societies should explore new means to leverage funding from government and private sources, to aid the development of new initiatives designed to strengthen the discipline.

9

Recommendations

1. Societies should advocate for continued funding of basic research and collect evidence to document its state in their country
2. Networks and working groups should be created, domestically, and internationally, by IUPS and member societies to facilitate the exchange of knowledge and best practice in teaching and research
3. Societies should continue the efforts of the IUPS Outreach Program to increase support among physiologists for IUPS initiatives and its furthering of the World Health Organization's Health for All agenda
4. Societies should implement outreach activities to raise awareness of and interest in physiology among the public and encourage the uptake of physiology and related subjects by prospective undergraduate and postgraduate students
5. Societies should develop resources to improve the teaching and learning of physiology, and to ensure graduates have a full appreciation of the complexities at all scales of physiological understanding
6. IUPS should oversee a new Global Mentorship Building Platform to facilitate Mentor/Mentee relationships among physiologists at various career stages, and in academic and clinical settings, to promote dialogue and aid career development
7. Societies should explore new means to leverage funding from government and private sources, to aid the development of new initiatives designed to strengthen the discipline
8. Physiologists across the globe should partner with a diverse pool of basic scientists and clinicians to spearhead research endeavors into identifying treatments and vaccines for the novel coronavirus that has caused the COVID-19 pandemic. The establishment of such an alliance may prove useful to more swiftly and efficiently counter future pandemics.

Essays

Vision

Artist: Anna Zeligowski, an artist and family doctor. In her pictures Anna presents a complex world of changing relations of men and women within an environment of plants and animals in myriads of colour that enhance and change seeing not only through words and concepts but also with instruments: we can see the very small and the very large and distant. Legend: Eva Jablonka, retired professor in the Cohn Institute for the History and Philosophy of Science and Ideas, Tel-Aviv University, a member of the Sagol School of Neuroscience, Tel-Aviv, and a Research Associate in the CPNSS (LSE, London University). Courtesy: Debabrata Ghosh, professor of Physiology at the All India Institute of Medical Sciences, New Delhi, India.

TRPV1: An Important Channel in Pain and Pain Modulation

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In light of substantial advances in the research on the pain and pain modulation in recent years, Williams and Craig accordingly proposed a much more comprehensive definition to "pain" as "a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive, and social components."[1] Since chronic pain is a long-term pathological change caused by nerve injury or abnormal nerve activity, it is a great challenge for modern clinical medicine and needs more research concerns. There are about one-fifth of the world's population suffers from chronic pain according to the statistics,[2] and about three-quarters of them have suffered from anxiety, depression, and other mental comorbidities of this disease. As a consequence, due to inadequate understanding of the mechanism of chronic pain and the reduced efficacy and side effects of analgesic drugs, patients of chronic pain could hardly receive timely and effective analgesic treatment, which has been always the significant issue for patients and clinics.

When dipping into the mechanism of sensitization in chronic pain, peripheral nociceptors are worthwhile targets for researchers due to their natural physiological characteristics. Peripheral nociceptors are distributed with a variety of receptors that can receive various forms of stimulation, such as cold, heat, chemical, and mechanical stimulation. These receptors constitute the material basis for temperature, chemical, or mechanical perception of the body. Among them, TRPV1, named as transient receptor potential vanilloid type 1, is highly expressed in the small and medium-sized primary sensory neurons involved in nociception. It is a nonselective, ligand-gated cation channel that contributes to the development of diverse types of pain such as inflammatory pain, bone cancer pain, migraine, irritable bowel syndrome, and arthritis. TRPV1 can be activated not only by heat (>43°C), acid (pH <5.9), capsaicin, and endogenous cannabinoid, but also be stimulated functional sensitization by local inflammatory mediators such as bradykinin, prostaglandin E2, NGF (nerve growth factor), and adenosine triphosphate, which layout a down-regulation of activation threshold and an increase both in channel current and membrane localization.[3] Based on the functional characteristics of TRPV1 mentioned above, it is called peripheral nociceptive information integrator because of its capability of integrating a various form of stimulation.

The reason for why TRPV1 could widely attract researcher's attention is that it takes part in the formation of nociceptive and pathological thermal hyperalgesia.[4,5] As their findings have revealed, the response of TRPV1 knockout mice to noxious heat stimulation was significantly reduced, especially in terms of the heat sensitization behavior in inflammatory pain mice model.[4,5] Even though TRPV2 comes from the same family of TRPV1, it is observed no abnormal thermal sensation in TRPV2 knockout mice.[6] These findings further strengthen the key role of TRPV1 in the formation of nociceptive thermal sensation and thermal hyperalgesia. Therefore, TRPV1 has been widely used as a molecular marker of thermal sensitive neurons in the dorsal root ganglion (DRG).

Sensitization is one of the most significant functional characteristics of TRPV1 channel. Its formation is regulated by many factors, among which phosphorylation plays an essential role. The phosphorylation and sensitization of TRPV1 induced by protein kinase A, protein kinase C (PKC), and CaMKII (Ca^{2+}/calmodulin-dependent protein kinase II) have been reported.[7] We have found out that TRPV1 could form complex with protein kinase D1 (PKD1) while there was no

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interaction between TRPV1 and other PKC subtypes were detected.[8] PKD1 was initially classified as a PKC family, known as the atypical PKC subtype. However, PKD1 is finally sorted as CaMK family because of its significant difference with other PKCs regarding its kinase characteristics and protein structure; as well as its high similarity with CaMK family in substrate specificity.[9] Our results demonstrated that PKD1 could not only bind to TRPV1 N-terminus but also phosphorylate its S116 directly, and this phosphorylation markedly increased the response of TRPV1 to capsaicin.[8] This study provided an essential basis for the research on the physiological functions of PKD1 and also laid a foundation for researchers in this field to explore further the functional regulation of PKD1 on TRPV1 and its possible role in inflammatory thermal hyperalgesia *in vivo*.

The subsequent work of our lab revealed that the over-expression of PKD1 in DRG neurons could significantly increase the thermal hyperalgesia of the inflammatory pain rat model induced by intraplantar injection of complete Freund's adjuvant. Instead, over-expressing the dominant-negative PKD1 (D727A-PKD1) or intrathecal injection of PKD1 antisense oligonucleotide could alleviate the behavior caused by the thermal hyperalgesia and simultaneously, no effect on mechanical hyperalgesia was found. In the DRG neurons of the inflammatory pain rat model, the co-localization on the cell membrane as well as the combination of PKD1 and TRPV1 was observed. Furthermore, enhancing the activity of PKD1 could not only raise the localization of TRPV1 on the cell membrane but also the current amplitude induced by capsaicin.[10] This finding first showed the interaction between PKD1 and TRPV1 *in vivo* and its effect on accelerating the inflammatory thermal hyperalgesia. Apart from PKD1, we have also drawn our attention to the cyclin-dependent kinase 5 (Cdk5), a unique member of the Cdk family expressed in the nervous system, has distinctive activator p35 and p39 (or its degradation product p25 and p29) rather than depending on the cyclin.[11] We first reported that Cdk5 was involved in the management of inflammatory thermal hyperalgesia and it is worthwhile to highlight that TRPV1 is one of its functional targets.[12]

TRPV1 is targeted by many kinases that influence its trafficking and activity. Considering the function of TRPV1 as a membrane receptor, not to mention that there were no findings reported the mechanism of TRPV1 membrane trafficking. We accordingly raised the interest in studying the possible mechanism of TRPV1 membrane trafficking as well as the regulatory role of Cdk5 in it. In detail, we proved that the kinesin-like protein 13B (KIF13B) participated in the TRPV1 membrane trafficking. Moreover, the Forehead-associated domain T506 could be phosphorylated by Cdk5, which promotes the binding of TRPV1 and KIF13B and the membrane localization of KRPV1.[13] In addition to the "Cdk5-KIF13B-TRPV1" pathway, we investigated the direct phosphorylation of TRPV1 by Cdk5. Results suggested that the phosphorylation of Cdk5 on TRPV1-T406 promoted the membrane localization of TRPV1, and the interfering peptide tat-TRPV1-T406 could reduce the membrane localization of TRPV1 and the response to capsaicin. Similarly, thermal hyperalgesia with inflammatory pain in rats could be alleviated.[14] Under further studies, we found that the actin polymerization induced by LIM motif-containing protein kinases (LIMK) in primary sensory neurons enhanced the response of TRPV1 to capsaicin in DRG neurons. These effects were reversed by the knockdown of LIMKs or preventing cofilin phosphorylation. Thus, LIMK-dependent action rearrangement was involved in the development of inflammatory heat hyperalgesia by leading to altered TRPV1 sensitivity.[15]

In recent years, we also revealed that Nogo-A, a critical inhibitory molecule of axon regeneration in oligodendrocytes, exerted its function in the development of inflammatory heat hyperalgesia by maintaining TRPV1 function through the activation of the LIMK/cofilin pathway.[16] Another study on the role of peripheral potassium channel interacting protein 3 (KChIP3) in inflammatory pain also revealed that the KChIP3-TRPV1 interaction reduced the surface localization of TRPV1 and therefore alleviates heat hyperalgesia and gait alterations induced by peripheral inflammation.[17] Studies on the internalization of TRPV1 have mainly focused on that induced by capsaicin or other agonists, and we showed that constitutive internalization of TRPV1 occurred in a manner dependent on clathrin, dynamin, and adaptor protein complex 2 (AP2). By phosphorylating the clathrin adaptor protein AP2μ2, Cdk5 played an inhibitory role on the clathrin-dependent internalization of TRPV1. This latest finding provides a detailed mechanism of TRPV1 internalization and a new potential target for clinical analgesic treatment.[18]

Since the efficacy of analgesic drugs in use for chronic pain, along with significant side effects, is not ideal enough, which limits its application in clinics widely. Hence, advancing the analgesic drugs in current clinical adoption as well as reducing

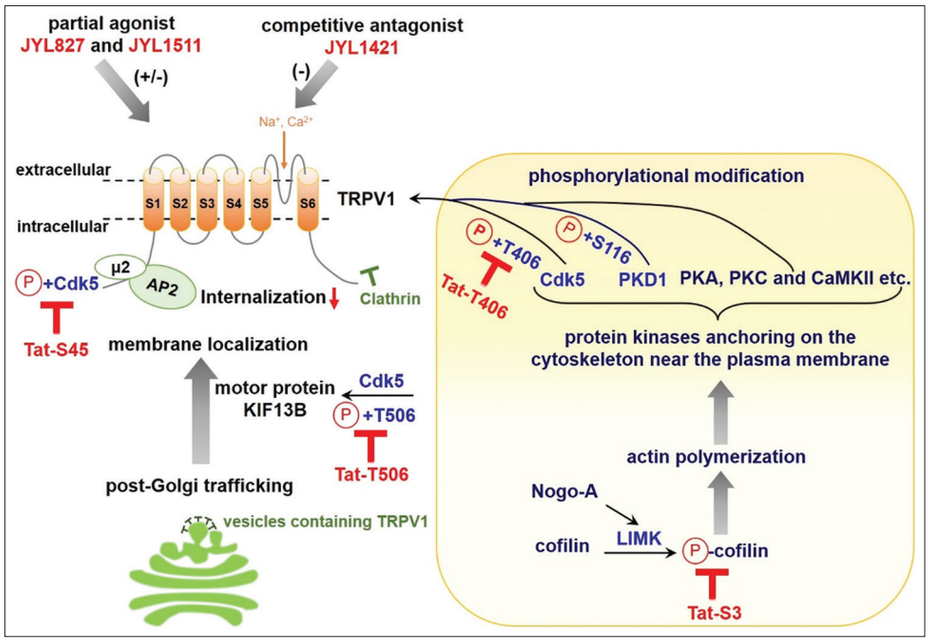

Figure 1: Summary of our research work on the phosphorylation regulation and membrane trafficking mechanism of TRPV1 in chronic pain in nearly two decades. The schematic describes the involvement of TRPV1 in pain and pain modulation by illustrating a series of our essential findings on the mechanism of sensitization and membrane localization of the TRPV1 as peripheral nociceptive information integrator.

its side effects is much more practical issues to tackle. For instance, enhancing the analgesic effect of capsaicin and morphine and weakening or eliminating the side effects of burning pain. Capsazepine, the most widely used receptor antagonist of TRPV1, has moderate action intensity and poor specificity as its disadvantages. To deal with these problems, we obtained two highly effective and specific competitive antagonists of TRPV1 receptor, KJM4229 and JYL1421 by the cooperation with Peter M. Blumberg Laboratory (National Cancer Institute, Bethesda, Maryland, USA).[19] Besides, two partial agonists of TRPV1 receptor JYL827 and JYL1511 were found. Once the pH value, temperature, receptor density or PKC activation level changes, the activation of TRPV1 receptor will be altered accordingly, or even turns into full agonists.[20] This phenomenon, on the one hand, has given clues and warms to the drug screening of TRPV1 receptor antagonists in the future, that is, the screening conditions such as pH value, temperature, and receptor density should be strictly controlled during the selection process. On the other hand, it suggests that some of the agonists may have potential clinical application prospects, and their effects could be easily altered with the environmental change of its sites of action under the inflammatory or some pathological situations. Thus, our research on exploring the agonists and antagonists of TRPV1 provides us with new possibilities and perspectives for the development of analgesic drugs targeting the TRPV1 receptor. Figure 1 is the summary of our research work on the phosphorylation regulation and membrane trafficking mechanism of TRPV1 in chronic pain in nearly two decades.

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The Molecular Physiology of Ion Channels: Past, Present, and Future Research

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Membrane proteins such as ion channels and receptors play critical roles in excitable and non-excitable cell and their functions are indispensable for life. In addition, there are many hereditary diseases caused by genetic abnormalities of ion channels and receptors, and our understanding of the molecular physiology of these proteins has greatly contributed towards understanding the pathophysiological mechanisms contributing to the disease and vice versa. Computational approaches, enabling quantitative and systemic analyses are increasingly contributing to our understanding of ion channel and receptor physiology. This compliments the more traditional biophysical approaches, utilized since the time of Hodgkin and Huxley, to elucidate the behavior of ion channels and thereby establish the basis of membrane excitability. In this article, I focus on the biophysical/molecular physiological aspects of ion channel research.

One of the most remarkable turning points of ion channel research was the isolation of cDNA encoding ion channels proteins by many labs.[1,2] Since then heterologous expression of ion channels was made possible, and also the analysis of structure-function relationships based on the systematic comparison of wild type and mutant proteins. These approaches provided us with invaluable information concerning the gating, ion permeation, and regulation of ion channels. Two noteworthy examples are the identification of voltage-dependent activation mechanisms[3] and inactivation mechanisms.[4]

Another remarkable advancement was provided by the analysis of crystal structures. As the crystallization of proteins embedded within membranes is not feasible, progress in this direction was initially slow. One of the most remarkable breakthroughs was the X-ray crystal structure analysis of the KcsA K^+ channel,[5,6] which contributed to solving the mechanisms of K^+ ion selectivity and permeation. Successful structural analyses of other membrane proteins followed. The two-dimensional/tubular crystal structure analyses using cryo electron microscopy for nicotinic ACh receptor[7,8] and Aquaporin[9] also progressed our understanding of ion channel structure.

This brings us now to consider the future of ion channel research and ask in what areas are breakthroughs likely that will define the next decade? In this article, I will look back at some of the past successes and consider recent methodological breakthroughs that are likely to provide the next major advancement in our knowledge.

Single Particle Structure Analysis by Cryo Electron Microscope (cryo-EM) Approaches

A major breakthrough took place in 2013, a high spatial resolution structure analysis of transient receptor potential vanilloid type 1 (TRPV1) channel by single particle analysis method using images obtained by cryo-EM.[10] It is a sort of in silico structure analysis, and the methodology itself has a long history. However, the spatial resolution was limited and the detailed structure could not be solved. The innovative changes involved with data acquisition and computation, as well as the usage of direct detection cameras for electrons, dramatically elevated the spatial resolution.

Cryo-EM structure analysis has several major advantages. (1) Structures can be solved without crystallization. As

the crystallization is a high hurdle for ion channel structure analysis, it truly changed the field of research and many structures have been solved since 2013. (2) Structures of membrane proteins in lipids can be solved with the use of lipid nanodisc.[11] (3) Structures of huge protein complexes can be solved, as evidenced by solving the structure of the mammalian respiratory super complex which is 1.7 M Da.[12] On the other hand, it is challenging to solve the structure of small proteins of <200 kDa by single particle structure analysis. Streptavidin, which is only 52 kDa, was solved by supporting on a thin layer of graphene and embedding in vitreous ice.[13] (4) Structures in multiple intermediate states can be solved simultaneously, dependent on the classification of particle images into these multiple substates.[14,15] (5) A recent advancement has enabled side chains to be visualized, for example in the recent atomic resolution imaging of the γ-aminobutyric acid type A ($GABA_A$) receptor.[16] This will make a valuable contribution to future drugs discovery efforts.

There is no doubt that the cryo EM single particle structure analysis has dramatically changed the field of ion channel research. We can soon hope for the structures of all major ion channels to be solved, and this information to be freely available, together with the homology modeling of the structures. Furthermore, with the accumulation of all this structural information, computational methods that use AI to predict protein structure based on the amino acid sequence will continue to be developed. Images with reasonable accuracy might be achieved in the not too distant future.

Molecular Identification of New Ion Channels and Accessary Subunits

Although the cDNAs of most of the major ion channels have already been isolated, there were some notable recent successes. The cell volume-sensing mechanism long remained elusive, and yet LRRC8 heteromers were finally identified as a volume regulated anion channel.[17] Besides the VSOP (Hv1) proton channel,[18,19] a totally new class of proton channel OTOP1 was isolated from mouse taste receptor cells.[20] Furthermore, the ATP releasing channel CALHM1 was isolated from taste cells[21] thereby highlighting a functional role for purinergic neurotransmission in taste sensing.

Ion channels often associate with other accessary subunits to form a functional molecular complex, such as Stargazin with the AMPA-type glutamate receptor channel.[22] The accessary subunits regulate the trafficking of the complex to the plasma membrane, ion channel gating, and pharmacological properties. An accessary subunit for the adenosine triphosphate (ATP) receptor channel P2X, TMEM163, was recently identified by screening of genome-wide open reading frame collections, which regulate functional properties such as desensitization.[23]

The stoichiometry of molecular complexes is of interest, and counting of subunits by the number of the bleaching steps of the attached fluorophore in single molecule imaging has been shown to be effective.[24] In the case of KCNQ1/KCNE1 channel complex, different results were obtained by single molecule imaging, suggesting either a 4:4 or 4:2 stoichiometry. A variable stoichiometry up to 4:4 was further supported by the recording of distinct single channel activities.[25]

Although the molecular identification of major ion channels has already been achieved, there are still those that are physiologically important that remain to be discovered. The identification of the components in the molecular complex will continue to be critical, including not only the membrane spanning accessary subunits, but also the anchoring proteins and extracellular proteins which contribute to the formation of synapses.

Regulation

Ion channel function is regulated by various biochemical mechanisms including phosphorylation, glycosylation, and palmitoylation. The regulation by lipids, such as phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), has also attracted considerable attention as (PI(4,5)P2) was shown to play critical roles in the regulation of KCNQ2/3 and also other channels. More generally, the constituents of the lipid bilayer are known to be critical for ion channel function. For example, the single channel conductance and gating of the KcsA channel was shown to change when the lipid in the inner leaflet was

the inner leaflet was changed, by lipid bilayer reconstitution experiment.[26] A new method called the contact bubble bilayer method was recently developed. In this method, water bubbles are blown into the oil phase from two glass pipettes, and lipid monolayer on the water bubble surface forms lipid bilayer when two bubbles are contacted. It enables a feasible control of the asymmetrical lipid bilayer constituents as well as the membrane curvature and tension.[27]

There are some unexpected mechanisms of ion channel regulation. KCNQ channel was shown to be directly activated by the inhibitory neurotransmitter GABA,[28] indicating that it is both a GABA- and voltage- gated channel. The ion selectivity and the inward rectification property of the ATP receptor channel, P2X2, changes depending on the expression density on the plasma membrane of P2X2 in the open state.[29] P2X2 also shows voltage-dependent activation on hyperpolarization, in spite the absence of a canonical voltage sensor in the molecule, and the conductance-voltage relationship shifts depending on the ATP concentration.[30] The regulation of ion selectivity by intracellular Ca^{2+} was recently shown for TMEM16F channel[31] and for TPC2 channel by the two physiological agonists, NAADP and PI(3,5)P2.[32] These recent finding indicate that the selectivity of ion channels is far more flexible and dynamic than was previously thought.

The activation mechanisms of thermo- and chemo-sensitive channels such as the TRP channels has attracted considerable attention.[33,34] The structures of TRPV3 at different temperatures were solved by quick freezing of the channel protein and cryo-EM structure analysis was used to investigate the mechanism of activation by heat.[35] The structure of mechanosensitive channels, such as ASIC1, Piezo, TREK/TRAAK, and NompC, showed a high level of variation indicating their divergent activation mechanisms[36,37] and is an area likely to continue to attract considerable attention.

Dynamic Aspects of Ion Channel Function

The structural analysis has provided us with a precise map, which contributes to the understanding of the functional mechanisms and also the design of mutants for further structure-function analysis. However, they are snap shots of still images and researchers have strived to see movies of functioning ion channels for better understanding of the dynamic structural rearrangements. Various new methodologies have been developed as follows.

1. Structures in multiple states: It is possible to solve static structures in multiple states. The structure of AMPA receptor in apo, antagonist, and agonist-bound states was solved by cryo-EM analysis, and by combining these images, the mechanism of channel opening was presented as a movie[38]
2. Diffracted X-ray tracking (DXT): In DXT, a gold nanocrystal is attached to a single channel protein and the movement of the diffraction spot of the attached gold associated with the tilting and rotation of the channel protein is tracked. By this method, the motion of KcsA channel during gating in response to low pH was visualized.[39] The scope of this method is expanding
3. X-ray free electron laser analysis of the real time structural rearrangements of proteins in the 3D crystal: It detects the triggered sequential conformation changes of protein in the 3D crystal. This innovative method was applied to the bacteriorhodopsin and the photonic stimulation evoked sequential structural rearrangements which were tracked in both the nanosecond and microsecond time range.[40] This is a straightforward method to see the movie of the functioning image in real time and at high spatial resolution. The limitation is that the observable motion is not a global but a rather local one which can occur in the 3D crystal, and that it is needed to trigger gating at a precise time. A further expansion of this application is desired
4. Atomic force microscopy: This method gives information of the shape of protein surface by scanning using a thin needle, that is, the height of each point in the scanned area. The time resolution was dramatically elevated and the method was successfully applied to membrane proteins such as bacteriorhodopsin[41] and F1-ATPase.[42] It is especially effective to observe the global structural rearrangements such as relocation of subunits. Further improvements in spatial and temporal resolution is desirable
5. Spectroscopic analyses 1: Electron spin resonance (ESR) tries to record the structural rearrangements using ESR

of introduced unpaired electron such as in NO, and has been applied to the structural rearrangements during activation of the KcsA channel.[43] DEER, the double electron-electron resonance technique by a double pair is used for better evaluation of the distance between the spins. It was applied to the conformational change of the cyclic nucleotide channel during gating [44]

6. Spectroscopic analyses 2: Voltage Clamp Fluorometry is a method to simultaneously record under voltage clamp the ion channel current and also the fluorescence intensity of the attached fluorophore. The basis is that the fluorescence intensity of the attached fluorophore changes in accordance with a change in its environment following a structural rearrangement. This method was applied to the analysis of the voltage sensor movement of Shaker channel,[45] KCNQ1/KCNE1 channel[46,47] and other channels. Forster Resonance Energy Transfer (FRET) analysis is frequently used to evaluate the distance and the change between the two fluorophores attached to the protein,[48] by recording the extent of the energy transfer between the two fluorophores. It is effective especially to detect a global conformational change and also a change in the relative location of two subunits. As the extent of energy transfer depends not only on the distance but also the angle of the orientations of the two fluorophores, the estimation of the physical distance is not feasible, and thus a method to use a lanthanoide such as Tb^{3+} as a donor was also used and known as LRET.[49] The bulkiness of the fluorophore is an issue especially when detection of fine structural changes is required. In tmFRET experiment, fluorescent unnatural amino acids (fUAAs) are used as a donor, and a transition metal ion such as Co^{2+} coordinated by two Histidines, or Cu^{2+}-TETAC labeled at Cys residue, is used as an acceptor. The tmFRET method was successfully applied to HCN channel to track the downward voltage-sensor movement upon hyperpolarization[50]
7. Usage of fUAA (supplement of [6]): fUAA can be used to introduce fluorescent label to the channel protein. This is a method to make fUAA incorporated into the target position in the protein at the time of protein translation, using an amber suppression codon introduced by mutation. An exogenous tRNA for the UAA and the modified ligase to link tRNA and UAA are expressed in advance and UAA is also introduced.[51,52] The advantage of this method are as follows. (a) UAA can be incorporated to any position in the protein. In the case of incorporation of maleimide- fluorophore using the introduced Cys residue, accessibility is critical, and thus inaccessible transmembrane and cytoplasmic regions cannot be labeled. (b) The labeling efficiency and specificity is high. If fUAA is not incorporated, it results in the termination of the translation of the channel protein. (c) The size is very small and can be incorporated to various positions without affecting the function. (d) The fluorophore is directly linked to the backbone of fUAA and thus it is possible to detect the subtle structural change of the backbone. As an example, fUAA method was successfully applied to the voltage-dependent structural rearrangements of Kv channel[53] and VSP (voltage-sensing phosphatase).[54] Besides fUAA, there are also other functional UAA which are convenient to use. One example is UAA which reversibly changes its structure by ultraviolet (UV) application and can therefore be used to induce forced conformational change by UV light.[55] Another example is UAA which induces photo-triggered crosslinking which is useful to stabilize a certain structural state at any timing of the photonic trigger[56]
8. Computational approach: Another effective approach to dynamic structural rearrangements is a computational approach by molecular dynamics simulation. The limitation was the relatively short time duration, but a relatively long simulation was performed and the motion of the voltage sensor in response to depolarization was shown.[57] The method is effective not only to resolve the local phenomenon such as ion permeation in the pore, but also global structural rearrangements associated with gating.

In this article I have briefly summarized past, present and future areas of ion channel research [Figure 1]. The advancement of the single particle structure analysis using cryo-EM has dramatically accelerated the rate at which high resolution structural information of ion channels has been obtained. This is not the ultimate goal, but it does provide us with a precise map for the journey. Science often advances with the development of innovative methodology and we hope in the near future to have dynamic images of functioning

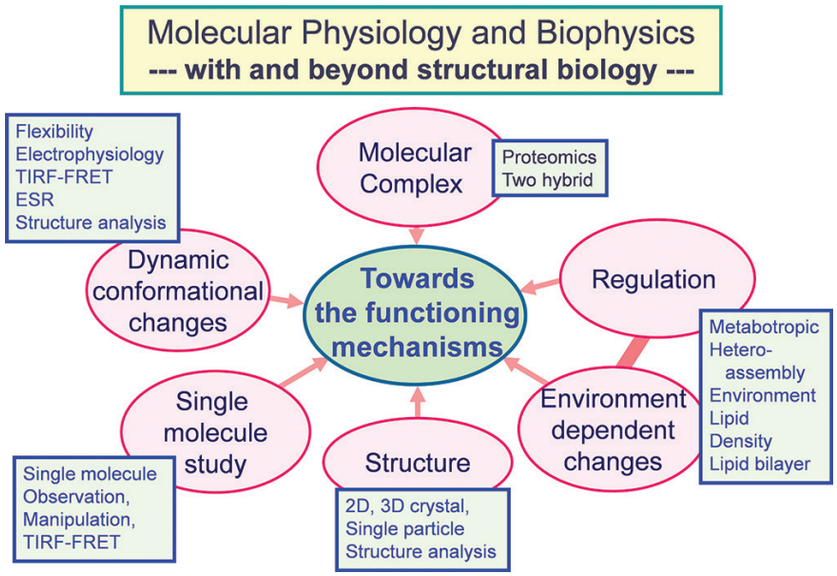

Figure 1: Perspectives of ion channel research.

ion channels that have both high temporal and spatial resolution.

Note: It was not possible to include all relevant achievements in this short article, and thus only a few representative examples have been cited.

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Current Research Regarding the Functions of Plant Homeodomain Finger Protein 2 in Diverse Physiological Progresses

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The architecture of eukaryotic chromatin plays a crucial role in the epigenetic regulation of gene expression; it is dynamically modulated by means of post-translational processes including acetylation, methylation, phosphorylation, and ubiquitination, of histone proteins at their N-terminal tails.[1] Among these modifications, the methylation of lysine residues in histones causes surrounding genes to be transcriptionally turned "on" or "off." For example, trimethylation of H3K4, H3K36, and H3K79 is a representative marker for active transcription. In contrast, mono-methylation of H4K20, di-/tri-methylation of H3K9, and tri-methylation of H3K27 are markers for silenced gene transcription.[2,3] Histone methylation is catalyzed by histone-lysine methyltransferases and is reversed by the histone-lysine demethylases (KDMs).[4] The KDM superfamily is divided into two families based on the properties of enzymatic reactions that they catalyze: Flavin adenine dinucleotide dependent amine oxidases and Fe2+/α-ketoglutarate-dependent dioxygenases.[5] The latter possess the conserved Jumonji (Jmj) catalytic domain. Thus far, more than 30 Jmj-containing KDMs (Jmj-KDMs) have been identified, most of which appear to function as demethylases or hydroxylases. Among KDMs, plant homeodomain finger protein 2 (PHF2)/KDM7C demethylates H3K9me2, which is a representative silencing marker in both facultative and constitutive heterochromatin architecture. We hypothesized that PHF2 would play an essential role in modulation of silenced gene transcription, depending on physiological and pathophysiological status. During the past decade, we have explored the role of PHF2 in diverse biological processes, including cancer progression, adipogenesis, osteogenesis, and neural function. In this report, we describe current research by our laboratory regarding the functions of PHF2.

Several genome-wide studies have demonstrated that the chromosomal region, including the PHF2 gene is deleted in many cancers, including colorectal cancer; therefore, we investigated the role of PHF2 in tumor suppression. PHF2 revealed to be associated with p53 as a transcriptional coactivator that promotes p53-driven gene expression through demethylation of the H3K9me2 repressive marker [Figure 1a]. On the basis of these findings, we concluded that PHF2 acts as a *bona fide* tumor suppressor in association with p53 during cancer development; moreover, it ensures p53-mediated cell death in response to chemotherapy.[6]

Several histone modifications are known to be involved in adipogenesis. Histone H3/H4 acetylation is associated with the activation of adipogenic gene expression[7] and H3K27 methylation through enhancer of zeste homolog 2 (EZH2) induces adipogenesis by silencing genes involved in the WNT pathway.[8] Knock-down of the H3K4/K9 demethylase LSD1 was found to represse adipogenesis; moreover, knock-down of the H3K9 methyl-transferase Setdb1 promoted adipogenesis in 3T3-L1 and 10T1/2 cell lines.[9] In hepatocytes, PHF2 is phosphorylated by protein kinase A and interacts with ARID5B to activate targeting promoters.[10] We also discovered that PHF2 is an essential epigenetic regulator in adipogenesis. PHF2 knockdown cells showed the lower lipid accumulation and metabolic pathway gene expression, compared to control cells. Notably, PHF2 physically interacts with CCATT/enhancer

binding protein (C/EBP)α and C/EBPδ as a transcriptional coactivator and epigenetically promotes the expression of target genes by demethylating the H3K9me2 repressive marker [Figure 1b].[11]

There is increasing evidence emerges that histone post-translational modifications elicit profound effects on synaptic plasticity, memory formation, and other long-lasting complex behaviors. For example, histone deacetylase inhibitors enhance the consolidation of associative memory in rodents.[12-14] The haploinsufficiency of euchromatin histone methyltransferase1 (EHMT1) is clinically associated with intellectual disability in Kleefstra syndrome;[15] loss of the Kmt2b gene in forebrain excitatory neurons induces impaired hippocampal memory formation in mice;[16] and LSD1+8a (a neuron-specific variant of LSD1/KDM1A) has been reported to participate in balancing memory formation and emotional behavior as a co-regulator of activity-evoked transcription of immediate early genes.[17] Accordingly, we discovered that PHF2 plays a critical role as an epigenetic regulator in memory formation. PHF2 is upregulated in the mouse hippocampus during the experience phase and is essential for memory formation. PHF2 interacts with activated CREB, serving as a transcriptional coactivator and epigenetically promoting the expression of memory-related genes by demethylation of H3K9me2. In behavioral tests with mice, memory formation was found to be enhanced by transgenic overexpression of PHF2, whereas it was impaired by silencing of PHF2 in the hippocampus. Electrophysiological studies have shown that PHF2 elevates field excitatory postsynaptic potential and N-methyl-D-aspartate receptor-mediated excitatory postsynaptic current in CA1 pyramidal neurons, suggesting that PHF2 promotes long-term potentiation [Figure 1c]. This research provides insight into the epigenetic regulation of learning and memory formation, thereby advancing our knowledge of memory improvement in patients with degenerative brain diseases.[18]

Notably, PHF2 mutation has been reported to cause dwarfism in mice[19] and PHF2 in differentiating thymocytes is correlated with expression of Runx2.[20] Further investigation is needed to determine whether PHF2 regulates Runx2-mediated bone formation. As predicted, overexpression of PHF2 facilitated bone development in newborn mice, while viral shRNA-mediated knockdown of PHF2 delayed calvarial bone regeneration in adult rats. In primary osteoblasts and C2C12 precursor cells, PHF2 enhanced osteoblast differentiation by demethylation of Runx2; conversely, its counterpart, suppressor of variegation 3–9 homolog 1 (SUV39H1) inhibited bone formation by methylation of Runx2. The interaction between Runx2 and the osteocalcin promoter is regulated by the methylation status of Runx2: Specifically, the interaction is augmented when Runx2 is demethylated [Figure 1d]. Thus, SUV39H1 and PHF2 reciprocally regulate osteoblast differentiation by modulating Runx2-driven transcription at the post-translational level. This research may provide a theoretical basis for the development of new therapeutic modalities for patients with impaired bone development or delayed fracture healing.[21]

For decades, it has been understood that histone acetylation and methylation must be balanced to maintain homeostasis. In the early 2000s, histone methylation was found to be a reversible reaction regulated by histone methyltransferase and histone demethylase, modulating repressive status depending on cellular context. Among histone modifications, H3K9me2 is decisive and representative silencing marker in most of fundamental biological processes. As the demethylase of H3K9me2, PHF2 acts to modulate and adjust repressive status according to cellular demand. As predicted, PHF2 was found to be associated with major representative transcriptional factors (e.g., p53, CEBP, CREB, and Runx2) in various physiological processes including tumor suppression, adipogenesis, glucogenesis, memory formation, and osteoblast differentiation [Figure 1]. Because PHF2 only possesses domains for protein-protein interaction and enzymatic activity, transcriptional factor harboring DNA binding domain must be recruited at the target gene promoter. PHF2 bound to a transcriptional factor behaves as either a transcriptional co-activator or a post-translational modifier, depending on cellular circumstances. In tumor suppression, adipogenesis and memory formation in the brain, PHF2 is a co-activator that cooperates with a representative transcriptional factor to enhance the expression of target genes through demethylation of the H3K9me2 repressive marker. Alternatively, in the context of osteoblast differentiation, PHF2 serves as a post translational modifier (i.e., non-histone demethylase) to demethylate Runx2; the subsequent removal of methylation from Runx2 augments the interaction between Runx2 and osteocalcin promoter to enhance osteoblastogenesis [Figure 1]. The mechanism that causes PHF2 to select one of these two actions remains unknown. Future research

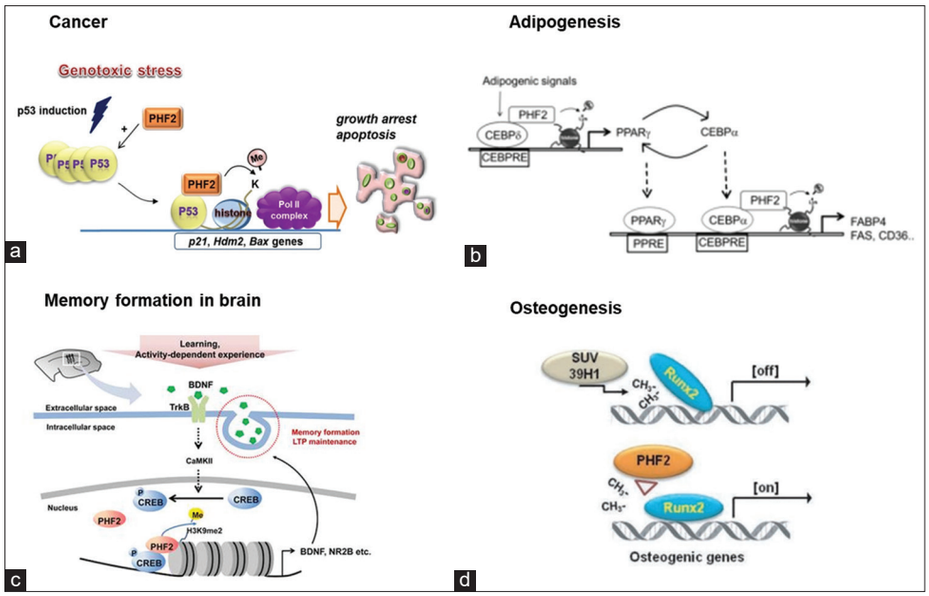

Figure 1: Schematic diagrams to explain how PHF2 play a role as transcriptional coactivators of p53 in cancer (a) CEBPα/δ in adipogenesis (b), and CREB in memory formation (c) by demethylating H3K9me2 of target genes. Intriguingly, PHF2 promotes Runx2 driven transaction in osteoblast differentiation by demethylating Runx2 at the post-translational level (d). For further details, see Kyung-Hwa Lee *et al.*,[6,11] Hye-Jin Kim *et al.*[18,21]

regarding applications of PHF2 may aid in development of new therapeutic targets for patients with cancer, metabolic disease, neurodegenerative diseases, and impaired bone development or fracture.

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The Blood-cerebrospinal Fluid Barrier as a Molecular Sieve: An Updated Approach

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Blood-cerebrospinal fluid barrier is a natural molecular sieve. It can be employed in basic and clinical research for diagnosis. The blood–brain barrier provides a complex, chemical-physical balance involving restricted bidirectional transport and energy consumption. It is the barrier that differentiates the composition of blood and of cerebrospinal fluid. To determine whether a substance originates in blood or cerebrospinal fluid, it is necessary to identify a protein that can serve as a marker whose bodily origin is known. This is fundamental to establishing the normal occurrence of proteins and other substances that can cross the blood–CSF barrier.

The protein that fulfills these requirements is albumin, which is synthesized, not in CSF, but in the liver. The albumin molecule, whose molecular characteristics are well defined, is transported from the blood to CSF. Q_{alb} — the CSF/serum albumin quotient — has proven to be the best indicator of the state of CSF barrier blood permeability.[1,2] Q_{alb} serves as the marker of CSF–blood barrier status, in that an increasing Q_{alb} in a given patient suggests a dysfunction of the barrier. This, in turn, may mean that more albumins is getting from blood to CSF or the speed of CSF flow during its circulation has slowed. It may also mean that the reabsorption of the CSF in subarachnoid areas has become slower to almost nonexistent, as happens during fetal life. There could also be a physical impediment, perhaps an obstacle due to a lesion or a tumor that occupies space in the circulatory path of the CSF. Alternatively, there may be an inflammatory process in some area of the CSF step, as happens in Guillain-Barré Syndrome.[3] Q_{alb} provides a baseline measure of normal albumin transfer and, by its comparison with other proteins, a means of determining the transfer and diffusion of these proteins from blood to CSF.[4]

Q_{alb} is a reliable numerical indicator of the passage of a protein from blood to CSF. If a molecule has a larger molecular weight than albumin, it will diffuse more slowly; if it has a weight, its diffusion is faster. The Q value of Q of a protein under normal conditions provides a measure of the proportion of that protein that diffuses through the CSF–blood barrier. At the same time, it enables determination of diffusion speeds or diffusion coefficients.

Applying the first and second Fick's diffusion laws,

$$J = -D\frac{dc}{dx} \text{ and } \frac{\delta c}{\delta t} = D\frac{\delta^2 c}{\delta x^2}$$

It is possible to determine the diffusion rate from blood to CSF, which depends on diffusion coefficient *D*. This diffusion constant of a protein depends on its molecular weight. Using the empirical distribution data of a protein from blood to CSF, its Q value has a hyperbolic distribution according with the Q albumin.

Comparing two proteins of different molecular weight [Figure 1], the only difference in the curves is the velocity of these proteins and their median displacement. The diffusion of a protein from the blood compartment to the CSF compartment, which can be obtained empirically, resembles a hyperbolic line. Figure 1 shows the general equation employed to perform the reibergram or Reiber quotient diagram.[5]

If one of the molecules is albumin, it is possible to determine the behavior of an unknown protein by comparison with its respective Q value. The reibergram was designed initially for the major classes of immunoglobulins,[6] but later reibergram determinations were employed for IgE,[7] IgG subclasses,[8] and proteins from different complement pathways, including C3c,[9] C4,[10] MBL,[11] and C5-C9 complex.[12]

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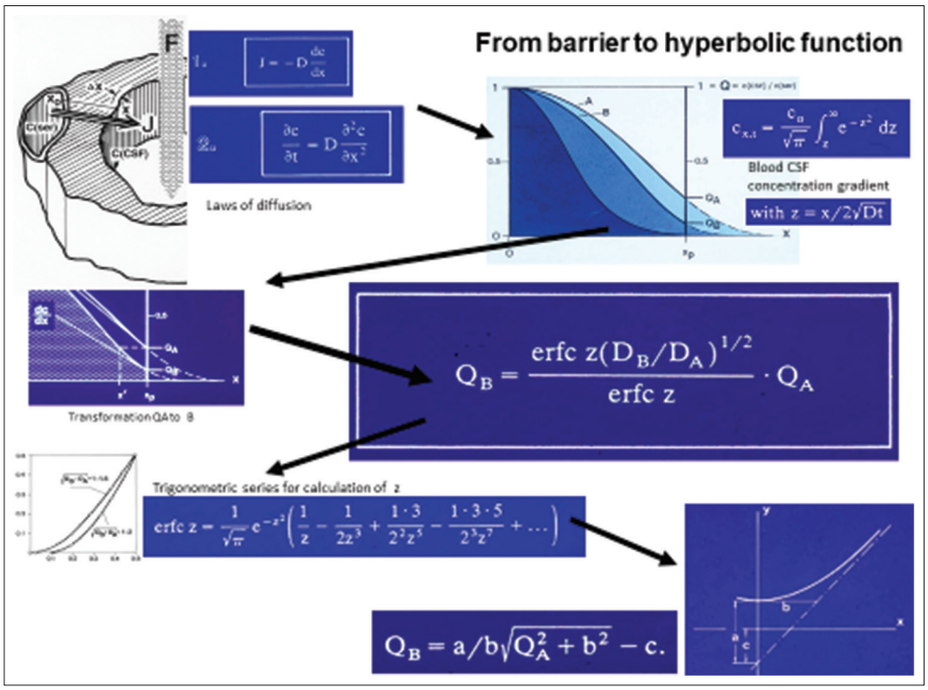

Figure 1: From barrier to hyperbolic function. For more details Reiber.[5]

Experimental Study: Molecular Diffusion of Substances from Blood to CSF

If the molecular weight of a substance is known, and the same is true of Q_{alb} and its molecular weight, one can calculate the normal passage of the protein from the blood to the CSF. The only variable that determines this is the value of its quotient. The value of the normal diffusion of a protein is given by its Q mean value obtained experimentally, that is, the normal relation of CSF and serum concentration.

It is possible to calculate the molecular weight of a given protein knowing the proportion of its diffusion between the blood and CSF compartments. This is done by comparing the molecular weight and normal Q_{alb} value with the normal Q value of the unknown, determined experimentally.

This calculation is done keeping in mind the hydrodynamic radius of the proteins, extrapolating the value of the hydrodynamic ratio or of the molecular weight against the values of normal Q for a group of known proteins as shown in Figure 2.[13]

Calculation of Polymeric Structures and Molecular Aggregation

Some molecules, including some components of the complement system, can be found in blood and in CSF in diverse polymeric form and in different aggregation states. Recognizing that the CSF–blood barrier acts as a molecular sieve, determining Q_{alb}, allows one to calculate the molecular weight of an unknown protein by comparing it with the behavior of albumin. After obtaining the Q

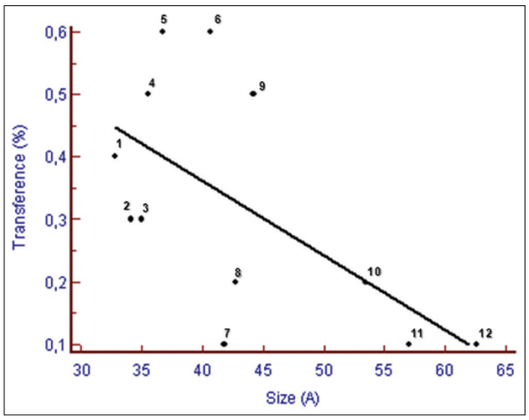

Figure 2: Variation of transference blood to CSF with the molecular size. (1) Alpha-1-antitrypsin; (2) Alpha-2 Hermann Schultz; (3) Hemopexin; (4) Albumin; (5) Transferrin; (6) Orosomucoid; (7) Haptoglobin 1-1; (8) Plasminogen; (9) Ceruloplasmin; (10) IgG; (11) IgA; (12) Haptoglobin 1-1 (with HB).

value experimentally by measuring the concentration of the unknown protein in CSF and serum pairs, it is possible to determine the number of monomers that comprise the polymer. This is done by dividing the experimental Q value by the expected Q value according to its molecular weight. Thus, knowing the polymeric form that crosses the membrane in comparison with both the expected and experimental Q values, it is possible to determine the aggregation forms that may be found relative to its Q albumin value. In this manner, it has been possible to calculate the natural polymer and the different aggregates of various components of the lectin pathway that cross the blood–brain barrier.[14-18]

Neuro-immunological and Clinical Applications

In basic neuro-immunology it is of value to determine the molecular weight of a protein that can pass through the blood–brain barrier. It is possible to determine the molecular weight of a protein knowing its Q value and be employed to determine the number of monomers that form in some structures that exist as both monomers and polymers. It is important to determine the number of aggregates of a polymeric structure for the activation of such proteins. It can be accomplished simply by measuring the albumin and the unknown protein in serum and CSF; not requiring complex instruments. Analyses employing reibergram determinations have been used to evaluate blood–brain barrier molecular behavior in other mammalian species.[19,20]

Q_{alb} has a variety of applications in neuro-immunology. Q_{alb} has a diagnostic value for Guillain Barré Syndrome,[3] where the experimental Q value is compared with the normal value by patient age.

Reibergrams permit the diagnosis of a variety of neurological diseases from the typical synthesis patterns of these disorders. The cerebral phase of African trypanosomiasis could be diagnosed in sub-Saharan Africa using reibergrams. Such screenings could prevent many deaths.[21,22] Determination of intrathecal synthesis patterns permits the establishment of a preliminary diagnosis and the initiation of effective treatment.[23-25]

Conclusion

Blood–brain barrier physiology can be effectively employed in both basic and clinical research for the diagnosis of many diseases based on the behavior of this barrier as a natural molecular sieve.

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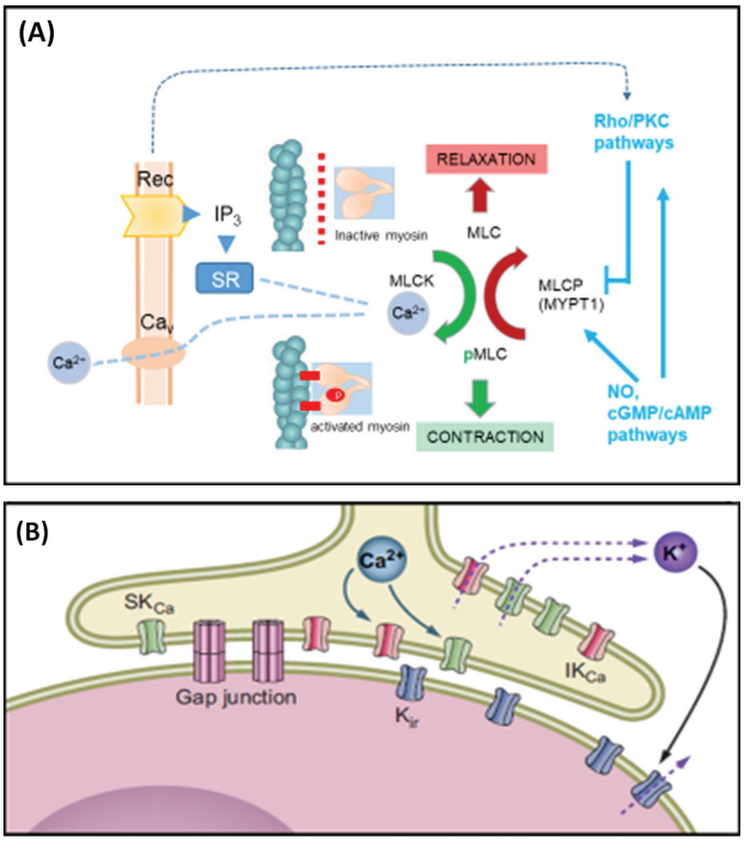

(A) Simplified scheme of mechanisms involved in smooth muscle relaxation- contraction. Increases in cytosolic Ca2+ activate the myosin light chain kinase (MLCK) which phosphorylates one of the light chains of myosin (pMLC) allowing actin-myosin interaction. Abbreviations: Cav: Voltage-gated calcium channel, Rec: G-protein coupled receptor, SR: Sarcoplasmic reticulum (calcium store), MLCP: Myosin light chain phosphatase. (B) Schematic diagram of endothelium (upper part) dependent hyperpolarization of smooth muscle cells (lower part) forming myoendothelial junctions (MEJ). MEJ are active sites exhibiting gap junctions as well as ion channels in their close vicinity. Local increases of intracellular free calcium (Ca2+) activate endothelial calcium-sensitive potassium channels (IKCa and SKCa), thus leading to potassium efflux and hyperpolarization of the endothelial cell. This hyperpolarization can be transmitted to the underlying smooth muscle cells by direct transfer of the hyperpolarization through MEJ and/or via the stimulatory effect of increase of the local extracellular potassium concentration on smooth muscle Kir channels that in turn elicits smooth muscle hyperpolarization. Hyperpolarization of smooth muscle cell membrane closes voltage dependent calcium channels resulting in intracellular free calcium which leads to smooth muscle relaxation and vasodilation (see A). B is taken from: U. Pohl Physiol Rev 100:525-572, 2020. Courtesy: Ulrich Pohl, Biomedical Center, Ludwig- Maximilians University, Munich, Germany.

The Smooth Way to Change – the Many Facets of Control of Vascular Smooth Muscle Tone

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Maintenance of adequate tissue oxygen supply in spite of rapidly changing demands is an enormous challenge for the cardiovascular system. Therefore, numerous neural, humoral, and local regulatory mechanisms act together on blood vessels to allow an actual moment to moment adaptation to the actual demand of the tissue.[1] The resistance vessels (arterioles) are the main target of regulation. According to Hagen-Poiseuille's law, the flow resistance of individual resistance vessels is determined by their internal radius (resp. diameter) which can be most effectively handled in vessels of this size. The arteriolar smooth muscles are normally in a state of partial contraction so that further vasoconstriction as well as vasodilation can effectively occur. This allows variation of blood flow over a wide range and, together with changes of the cardiac output, also the control of arterial blood pressure. All cells of the microvascular wall, including endothelium and adventitial cells, play an important role in regulating vascular diameter.[2,3] However, the microvascular smooth muscle remains the central effector since its state of contraction, the vascular tone, finally determines the vascular diameter. This short essay aims to highlight mechanisms controlling smooth muscle contraction and relaxation and their complex interaction.

The vascular smooth muscle is well adapted to its tasks. Most of the time it must maintain considerable tension to oppose (together with the vascular matrix) the distending force of intravascular blood pressure. This contraction can be brought about continuously and at a low energy cost.[4] Due to the different stoichiometry and arrangement of actin and myosin,[5] vascular muscle can shorten more extensively than striated muscle so that vessels can even be completely closed if necessary.

The state of smooth muscle contraction – the vascular tone – was originally thought to be exclusively regulated by the cytosolic concentration of free calcium ions. In fact it is the major determinant of the moment to moment diameter regulation of resistance vessels. Early studies revealed that an increase of the concentration of calcium ions leads to their enhanced binding to the acceptor protein calmodulin. The newly formed calcium-calmodulin complex activates the enzyme myosin light chain kinase (MLCK) resulting in an enhanced phosphorylation of the 20-KD regulatory light chain (MLC20) attached to the myosin heads. Phosphorylation allows the myosin to interact with actin, starting cross bridge cycling, which is the basal mechanism underlying muscle shortening.[6-8]

As pointed out, the phosphorylation state of MLC20 controls the cross bridge cycle, and hence, the acute smooth muscle contraction. It is not only influenced by the (calcium dependent) activity of the MLCK but also by the activity of its counterpart, the myosin light chain phosphatase (MLCP). A shift of the balance toward MLCK will increase, toward MLCP will decrease the tone. An altered phosphatase activity will relax or contract smooth muscle even when the calcium level remains unchanged. Since contraction or relaxation occur here without a change in cytosolic calcium concentration, the apparent calcium sensitivity of the contractile apparatus is changed. MLCP is under the control of the regulator proteins myosin phosphatase target subunit 1 (MYPT1) and the PKC-activated PP1 inhibitor protein (with Mw of 17 kDa, CPI-17) which exert autoinhibition and/or activator dependent inhibition of MLCP.[9,10] Mainly PKC and the RhoA dependent activation of Rho associated protein kinase (ROCK) can phosphorylate these regulatory proteins,

though possibly at different times and to a different extent in various types of vascular smooth muscle.[9] This calcium sensitivity pathway is often activated simultaneously by receptor dependent signaling pathways that increase calcium. Indeed a "calcium dependent calcium-sensitization" involving PKC was suggested.[9] This has the advantage that the contraction can be acutely enhanced. Moreover, this crosslink between signaling pathways has the potential to maintain constriction without the need of constantly elevated calcium concentrations. These may otherwise potentially affect other cellular signaling pathways at long last.

In addition to acute changes of tone by altered cross bridge cycling, smooth muscle cells can adapt their spatial arrangement[11] and their stiffness[12] in order to maintain a certain vascular diameter without ongoing contraction. The latter is achieved by plastic changes particularly of the actin cytoskeleton and of related membrane adhesion complexes.[12] Although such changes of the cytoskeleton can occur within minutes, they probably act also on a longer time scale and contribute to the remodeling of blood vessels as observed under chronically elevated blood pressure.[13] Thus, vascular smooth muscle cells have a multitude of mechanisms which do not only allow to change vascular diameters acutely but also to maintain such changes by plastic remodeling of smooth muscle cells.

Alterations of smooth muscle tone or size can be induced via many receptors and/or activation of ion channels.[14] They cannot discussed here in full detail. In the following I will therefore restrict myself to some illustrative examples.

One of the most significant properties of vascular smooth muscle is the "myogenic response" to changes in transmural pressure.[15,16] It is not only responsible for the maintenance of a basal tone of microvessels on top of which vasoconstrictors or dilators can act in addition. It is also the major basis for the autoregulation of blood flow following changes of blood pressure as first observed by Bayliss.[15] In general, pressure induced stretch of microvascular smooth muscle cell membrane results in a cation influx, elicited by a signaling cascade involving stretch sensitive receptors,[17,18] opening of cation channels of the TRP channel family and secondary activation of voltage dependent channels (T- and L-type, $Ca_v3.1$, $Ca_v3.2$, and $Ca_v1.2$) by depolarization of the vascular smooth muscle membrane.[14] Since the extracellular calcium concentration exceeds the cytosolic concentration within a resting cell by a factor of roughly 10^4, calcium will diffuse into the cell whenever calcium channels open. This leads to an influx of extracellular calcium activating and maintaining vasoconstriction as described above. The latter can be supported by a concomitant calcium sensitization through inhibition of myosin phosphatase as pointed out before. The interaction of several pathways may also explain that not all components of the pathway play the same role in different vascular beds.[9]

The smooth muscle membrane not only contains Ca_v and TRP channels but also a plethora of different potassium channels (for details[14]) whose activation generally induces membrane hyperpolarization and closure of voltage dependent calcium channels.[14] This reduction of calcium influx shifts the balance towards MLCP thus reducing MLC20 phosphorylation which results in vasodilatation. Such a response acts either as negative feedback mechanism limiting the effect of vasoconstrictors and the myogenic response,[19] or mediates the response to vasodilator compounds. An activation of smooth muscle potassium channels (K_{IR}, inward rectifying potassium channels) plays also an important role in vasodilation of blood vessels in working skeletal muscle or in active areas of the brain.[1,20,21] Endothelium derived vasodilation is partly also induced through activation of potassium channels: Specifically in the microcirculation, the vascular endothelium shares contact areas with underlying smooth muscle cells ("myoendotelial junctions," MEJ). These MEJ, containing gap junctions, and areas with high channel density[22,23] are an integral part of the cascade leading to the so called "endothelium dependent hyperpolarization" formerly defined as endothelium derived hyperpolarizing factor response.[24] Some vasodilatory prostaglandins[25] as well as cytochrome 450 products[26] or H_2O_2[27] can also act through smooth muscle potassium channel activation. Therapeutically, calcium entry blockers which inhibit Ca_v are widely used for treatment of hypertension targeting smooth muscle relaxation in resistance vessels.

While these local mechanisms play mainly a role for local regulation of blood flow, the sympathetic nervous system can affect many vessels simultaneously thereby changing the total vascular resistance (peripheral resistance). The smooth muscles of resistance vessels express alpha 1 and alpha 2 receptors which can bind the sympathetic neurotransmitter,

norepinephrine. Activation of the receptors leads either to IP3 mediated calcium release from intracellular stores ($alpha_1$[28]) or a decrease of the activity of adenylate cyclase and a G_i- dependent reduction of the vasodilator action of the second messenger cAMP ($alpha_2$[29]). In both cases, vasoconstriction occurs. Interestingly activation of $alpha_1$ receptors leads also to inhibition of MLCP thereby enhancing the vasoconstriction by augmenting the calcium sensitivity.[9] Numerous other vasoconstrictors, for example, locally produced endothelin or platelet-derived serotonin can further induce local or systemic vasoconstriction.

The increased calcium sensitivity as induced by norepinephrine but also by other vasoconstrictors often includes activation of Rho A/ROCK and the PKC signaling pathways inhibiting phosphatase activity. Conversely, vasodilators can inhibit the RhoA PKC activation or target MLCP associated proteins directly.[9] Consistent with this we observed that the guanylate cyclase stimulator NO induced vasodilation without affecting the calcium level but reducing calcium sensitivity.[30] It has been shown that NO can affect through its second messenger cGMP and activation of G-kinase also myosin phosphatase activity.[31,32] This is mainly achieved through inhibition of Rho-Kinase and PKC. A direct, cGMP dependent regulation of MYPT1 appears also possible but requires the expression of an isoform of MYPT1 which is not always expressed in microvascular smooth muscle.[31] Of note, NO/CGMP has been shown to affect also the cytosolic calcium level by interfering with IP3 receptors through IRAQ to reduce at calcium release from intracellular stores and inhibiting Ca_v channel activity while increasing the activity of certain potassium channels.[14]

Long-term exposure of isolated microvessels to norepinephrine for several hours elicits only initially calcium increase and MLC20 phosphorylation.[13] In spite of a normalization of the calcium concentration and later reduction of MLC20 phosphorylation, the constriction is maintained. This can be explained by the cytoskeletal remodeling already mentioned but also by a re-arrangement of smooth muscle cells around the vessel lumen.[11,13] As a consequence of the re-arrangement of cells, the vessels are not able anymore to fully dilate to control diameters. This can be understood as a mechanism to unload the contractile machinery by "resetting" the diameter control. This is associated with changes in the smooth muscle adhesion points, resulting in activation of integrin signaling which has been shown to have additional effects on smooth muscle tone.[33] Later on this remodeling is completed and stabilized by concomitant changes of the extracellular matrix.[13]

The different mechanisms of smooth muscle control shortly highlighted here should not be considered working independent of each other. Rather they represent an orchestrated response which occurs in part sequentially within time domain allowing fast and long-term responses where appropriate. An example for the multimodal effects of vasoactive agents is evident under hypoxia. Hypoxic tissue–derived signals such as urocortin2 can, for example, stimulate the enzyme AMPK-related kinase (AMPK) in vascular smooth muscle.[34] This enzyme was originally characterized as a cellular energy sensor acting in liver and muscle cells.[35] AMPK has not only been found to control smooth muscle calcium homeostasis acutely by activating (BK_{Ca}) potassium channels and the calcium pump SERCA (through phosphorylation of Phospholamban)[36] but also by reducing the cytoskeletal F-actin.[34]

Given the diversity and plasticity in the control of smooth muscle tone it seems necessary to apply these insights to our therapeutic strategies, for example, of hypertension. It is possible that current therapeutic approaches to interfere with vascular tone in hypertension therapy may benefit from more complex strategies as opposed to current strategies targeting single mechanisms such as calcium entry blockade.

This short essay hopefully illustrates that our knowledge about regulatory mechanisms in vascular smooth muscle are continuously expanding. Nevertheless, this area represents only a small sector of the broader field of vascular physiological research comprising vascular growth, vascular permeability, vessel coordination, or their interaction with blood cells, to mention only a few.

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"Trekking in the Khumbu Valley, on our way to the Pyramid Research Centre near the Everest Base Camp in Nepal. Everest can be seen In the distance". Courtesy: Federico Formenti, King's College London, and University of Oxford, Oxford, United Kingdom.

On Oxygen in Respiratory, Clinical, and Exercise Physiology

Federico Formenti

King's College London, and University of Oxford, Oxford, United Kingdom.

Our research encompasses three main areas: Respiratory physiology for intensive care medicine, human responses to hypoxia, and exercise physiology. A large variety of techniques is typically required to answer our research questions, so we have a range of technical skills for integrative physiology and a number of outstanding collaborators, each of which contribute to this multidisciplinary work.

Respiratory Physiology for Intensive Care Medicine

We measure arterial partial pressure of oxygen as an index of overall pulmonary ventilation and perfusion in the mechanically ventilated lung. This continuous oxygen monitoring is performed with an intra-arterial, fiber optic, and fast responding oxygen sensor,[1,2] which affords the real-time study of dynamic cardiopulmonary physiology, providing detailed information on pulmonary gas exchange [Figure 1, LEFT].[3-5]

The key advantages of this technology are that it is sufficiently small to fit into a small artery through a standard arterial catheter, it responds to oxygen changes rapidly,[6] so it can track within-breath changes, and it can be used in humans. The main disadvantage is that on its own, in the absence of an accurate model of the cardiopulmonary system in the context of lung injury, the sensor is currently unable to distinguish between changes determined separately by pulmonary ventilation and perfusion. To overcome this limitation, we study pulmonary ventilation and perfusion with dual-energy computed tomography [Figure 1, MIDDLE] and electrical impedance tomography [Figure 1, RIGHT], where spatial and time resolution are the respective strengths.[4,5,7] This combined approach allows the experimental dynamical investigation of arterial oxygenation and its main cardiopulmonary determinants, for example, tidal pulmonary blood redistribution.[4] Our novel oxygen sensing technology, together with the experimental evidence from our studies will then inform advanced physiological models of the cardiorespiratory system in humans with lung injury. We hope that this research may contribute to the care of mechanically ventilated patients in the intensive care unit[8] and in the field of veterinary anesthesia.

This research is led by Dr. John Cronin, Dr. Douglas Crockett, Dr. Minh Tran, and Dr. Joao Batista Borges, in collaboration with Dr. Luigi Camporota, Dr. Andrew Obeid, Dr. Joao Soares, and Professors Andrew Farmery, Clive Hahn, Anders Larsson, and Göran Hedenstierna. Our work in this field is sponsored by the NIHR, MRC, NIAA, the University of Oxford, the University of California Davis, King's College London, and the Physiological Society of London.

Human Responses to Hypoxia

We explore how a protein called hypoxia-inducible factor is involved in the regulation of human responses to hypoxia. We observed that mutations affecting the hypoxia-inducible factor pathway can have an impact on human respiration[9] and metabolism[10] at whole body level, with implications for high altitude training. In particular, we have confirmed some of the physiological responses (predicted from cellular models) at the level of the whole organism. This human physiology work was based on evidence from cellular experiments demonstrating the role of the hypoxia-inducible factor on a very large range of cellular and organ functions, for example, the production of erythropoietin, denser microcirculatory network, and the metabolic changes in hypoxic conditions. These models have implicit limitations in terms of improving our understanding of integrative physiology at the level of the whole organism.

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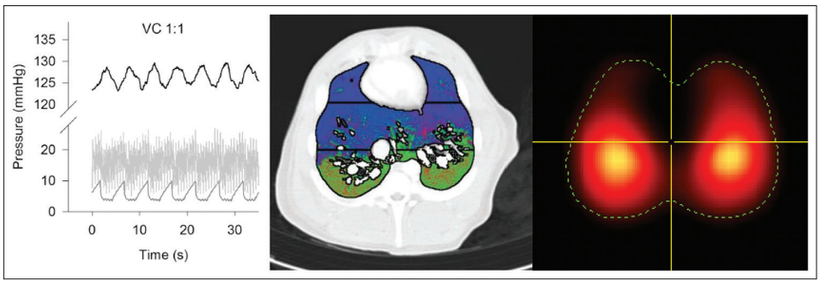

Figure 1: LEFT: PaO_2 oscillations in the uninjured, ventilated lung. Representative continuous measurements of PaO_2 (top and black), pulmonary artery pressure (middle and light grey), and airway pressure (bottom and dark grey) are presented as a function of time. Ventilation was managed in volume control mode, with inspired-to-expired ratio was 1:1. MIDDLE: A dual-energy computed tomography image of a juxtadiaphragmatic slice of pig's thorax during iodine infusion. The image shows lung parenchyma with the three gravitational regions of interest displayed: Gas (blue), soft tissue (green), and iodinated blood (red) volume fractions. RIGHT: Representative regional distribution of pulmonary perfusion as recorded by electrical impedance tomography in a piglet. The lighter red indicates greater perfusion than darker red, with yellow indicating the greatest perfusion. The dotted line shows the contour of the corresponding ventilation map (i.e. the corresponding pulmonary ventilation area studied at the same point in time). Images adapted from.[3-5]

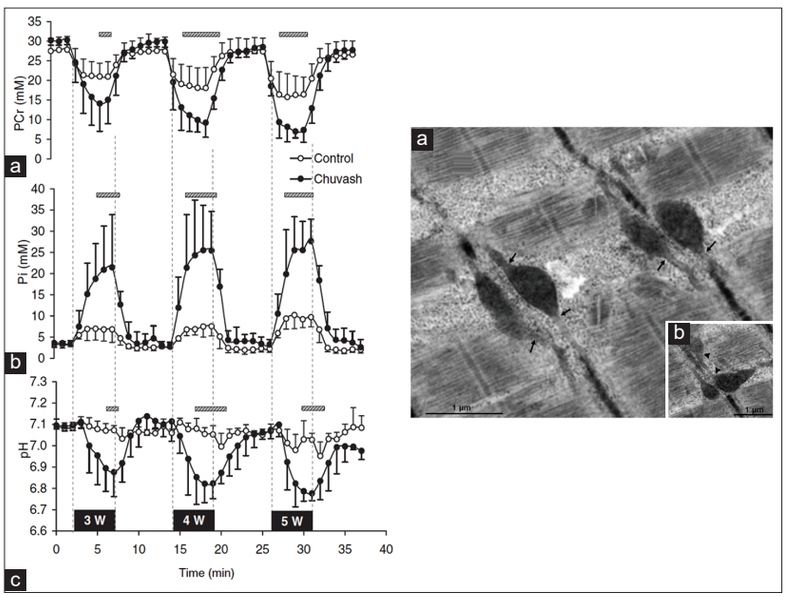

Figure 2: LEFT: Results from patients' group (Chuvash) and control group for 31P magnetic resonance spectroscopy on calf muscle: (a) phosphocreatine concentration, (b) inorganic phosphate concentration, and (c) pH as a function of time. The vertical broken lines indicate the onset and offset of the 5-min plantar flexion exercise sessions, and the associated black bars indicate the power outputs (3, 4, and 5 W). Empty circles show results from the control group (n = 5); filled circles show results from the Chuvash polycythaemia group (n = 5). Values are minute averages ± SD. Hatched horizontal bars indicate periods of significant difference (P < 0.05) between groups. RIGHT: Intermitochondrial ducts in the muscle from a patient with a mutation leading to reduced levels of von Hippel–Lindau protein. (a) Mitochondria from a vastus lateralis biopsy.[13] (b) Elongated profiles are patently connected to mitochondria at either end. Images adapted from.[12]

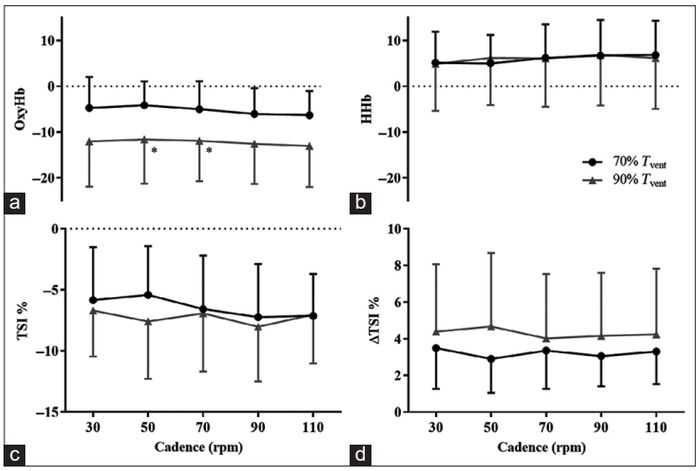

Figure 3: Skeletal muscle oxygenation indices at different exercise intensities and cycling cadences. Mean and standard deviation values: (a) OxyHb, (b) HHb, (c) TSI, and (d) Delta-TSI for each cadence (30, 50, 70, 90, and 110 rpm) during the tests at 70% of the ventilatory threshold (black symbols) and 90% of the ventilatory threshold (gray symbols). n = 12. TSI changes from baseline and Delta TSI are presented as percentage values; OxyHb and HHb are presented in arbitrary units and shown as changes from baseline. *P < 0.05 when compared to the same cadence at 70% of the ventilatory threshold using the Paired samples t-test. TSI, Tissue Saturation Index; Delta TSI, amplitude of TSI cyclical oscillation within pedal revolution; Hb, Hemoglobin; OxyHb, Oxygenated Hb and myoglobin; HHb, Deoxygenated Hb and myoglobin; Tvent, Ventilatory threshold; rpm, revolutions per min. Image from.[15]

We demonstrated in patients with a mutation in the hypoxia-inducible factor pathway that muscles have limited aerobic exercise capacity, associated with a reduced capacity to utilize oxygen at the muscle level [Figure 2, LEFT]. We also identified other abnormalities, for example, in neutrophil function,[11] in the physiology of the cardiopulmonary and endocrine systems, and in mitochondrial structure [Figure 2, RIGHT][12] and function.[13]

This research is performed in collaboration with Dr. Domenico Roberti, Professor Peter Robbins, Keith Dorrington, Fulvio Della Ragione, Silverio Perrotta, Andrew Murray, Clara Franzini-Armstrong, Manuela Lavorato, Roland Fleck, and Leanne Allison. Our work in this field was sponsored by the Wellcome Trust, the University of Oxford, King's College London, and the Physiological Society of London.

Exercise Physiology

We study the effect of different pedaling rates on the oxygen uptake of cycling exercise, showing that the work required to spin the lower limbs can be a substantial determinant of overall oxygen uptake.[14] We further investigated skeletal muscle oxygenation in the *Vastus laterialis* muscle in response to different cadences and work rates,[15,16] showing how it can decrease only marginally with increased cadence at moderate work rates in participants with a wide range of exercise capacity and cycling expertise, and how it is more greatly affected by increased work rate [Figure 3].

This research is typically led by undergraduate and graduate students, in collaboration with Professors Koji Ishida and Gerrard Rafferty. Our work in this field is sponsored by King's College London and the Great Britain Sasakawa Foundation.

Acknowledgments

We thank our participants for their contributions, and the Councils, Institutes, and Universities that fund our research.

Throughout all this and previous work, I was blessed with truly fantastic supervisors, colleagues, and students, to whom the

credit for the work goes, and to whom I am very grateful. And I must surely thank my wife and my family for supporting and allowing my work, but especially for reminding me to choose a healthy balance between work and the most valuable family time.

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Physiological Approaches to Modification of Mesenchymal Stromal Cells ex vivo: Focus on Tissue-Related Hypoxia

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Over the two past decades, significant experimental background from numerous *in vitro* studies of multipotent mesenchymal stromal cells (MSCs) has been accumulated. These data have given a reason to hypothesize on the mechanisms of MSCs involvement in physiological and reparative remodeling. The production of biologically active metabolites (cytokines, extracellular vesicles, adhesion molecules, extracellular matrix components, etc.) by MSCs is assumed to provide a specific microenvironment for various cell populations. At the same time, the MSCs are influenced by cellular and non-cellular tissue niche factors. The low O_2 tension being the most important physical ones. It is quite obvious that the O_2 level can play a crucial role in the implementation of MSC potency. The effects of low O_2 on the MSC functions have been intensively studied over the past 15 years, as evidenced by year to year increase of publications, the results of which have been summarized in several reviews.[1-4]

After short hypoxic exposures, both damaging and stimulating effects were demonstrated on MSCs [Figure 1]. It is known that this cellular response leads to the activation of a number of stress-induced signaling pathways, such as cascades of mitogen-activated protein kinases. Having high plasticity, MSCs constantly cultivated at 20% O_2 quickly adapted to the short-term O_2 deprivation, significantly changing their functional activity. In particular, their ability to migrate and stimulate angiogenesis has been shown to increase.[1,2]

Implementation of permanent expansion of MSCs at tissue-related O_2 levels is attracting an increasing interest. Our laboratory was among the first ones to widely use this experimental approach. A comprehensive analysis of the functional activity of MSCs from adipose tissue stromal vascular fraction (ASCs) under "physiological" hypoxia (5% O_2), that is, at an O_2 closely related to which MSCs exist in tissue niches *in situ*, revealed a number of features that can determine the efficacy of the involvement of these cells in physiological and reparative remodeling processes [Figure 2].

The transcriptional profile of ASCs under "physiological" hypoxia could be described as "pro-regenerative," providing the realization of functions associated with microenvironmental remodeling [Figure 3].

Indeed, the functional activity of ASCs changed significantly at 5% versus the ambient 20% O_2: The cell population grew faster, while the sensitivity to osteo- and adipo-stimulation was decreased. Such a shift in the ratio of proliferation/differentiation indicates a lower degree of ASC commitment under "physiological" hypoxia, which is also confirmed by an increased transcription of *DMKN*, *LAMA1*, and *GPR56* genes known to be associated with MSC "plasticity."[5]

Analysis of ASC paracrine activity which is currently considered as the most important mechanism for realization of the stromal precursor potential is of particular interest. After a short-term hypoxic exposure of MSCs expanded under standard 20% O_2, both an increased transcription and translation of a number of the most important immunomodulatory, pro-/anti-inflammatory, hematopoiesis-supporting chemokines were found.[6] In our experiments,

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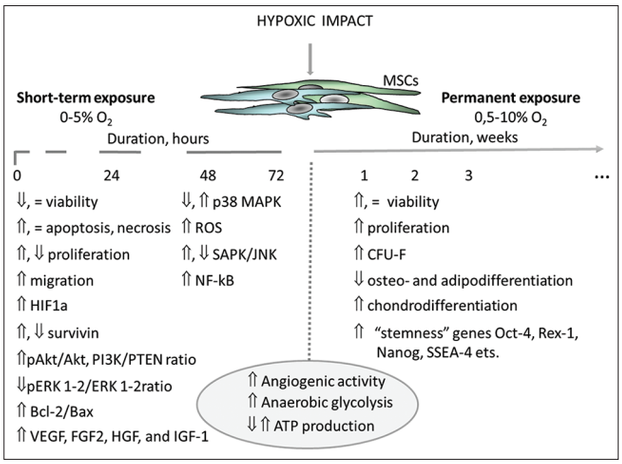

Figure 1: Schematic summary of short- and long-term hypoxic effects on MSCs. The cellular response to short-term exposure is in some extent controversial, displaying both hampering and stimulating outcomes. Permanent expansion under hypoxia provides maintaining of low differentiated state of MSCs. In filled frames, the phenomena common for both impacts are highlighted. Adapted from Buravkova *et al.*[1]

the objective was to evaluate the paracrine potential of ASCs continuously cultured at O_2 related to that of the relevant tissue niches. The whole genome analysis of ASCs under "physiological" hypoxia demonstrated a significant upregulation of genes encoded pleiotropic mediators such as MCP-1, IL-1b, IL-8, and LIF. However, the levels of these and a number of other cytokines and chemokines in the ASC-conditioned medium were similar at 20% and 5% O_2. At the same time, the ASC-conditioned medium at 5% O_2 primarily stimulated the growth of the vessels in the chorioallantoic membrane of Japanese quail embryo, which suggests the production of some additional, still unidentified pro-angiogenic factors at "physiological" hypoxia.[7]

Based on the above data, we assumed that a modification of ASC properties under hypoxia may have effects on their interactions with other types of cells, differentiated, or stem hematopoietic cells, in particular.

The evaluation of immunosuppressive potential of ASCs on allogeneic stimulated peripheral blood mononuclear cells (PBMCs) revealed significant antiproliferative effect on T cells, as well as suppression of T-cell activation manifested as a attenuation of HLA-DR expression, a lymphocyte activation marker. We were able to demonstrate a more pronounced immunosuppression on direct intercellular contacts, which was further potentiated under hypoxia [Figure 4].[8,9]

Interaction with activated PBMCs provoked a transcriptomic profile shift in ASCs: 304 genes at 20% and 142 – at 5% O_2 were differentially expressed with only 104 genes jointly changed [Figure 5a]. Ranking according to GO demonstrated that in groups of signal transduction, proliferation, immune response, cell adhesion, stress response, intracellular signal transduction, and cell motility, 20 or more genes were differentially expressed under 20% O_2. At 5% O_2, the number of genes with altered expression in the same groups was lower. In some GO groups, the differentially expressed genes were detected only at 20% O_2 (cell homeostasis, protein biosynthesis, and intracellular transport) [Figure 5b]. More detailed analysis demonstrated that interaction of ASCs with PBMCs under ambient O_2 as well as at "physiologic" hypoxia resulted in "priming" of ASCs with significant upregulation of genes involved in pro-inflammatory activation, immunosuppression, cell proliferation, cytokine regulation, and extracellular matrix remodeling.

ASC coculture with umbilical cord blood hematopoietic stem and progenitor cells (HSPC) under "physiological" hypoxia

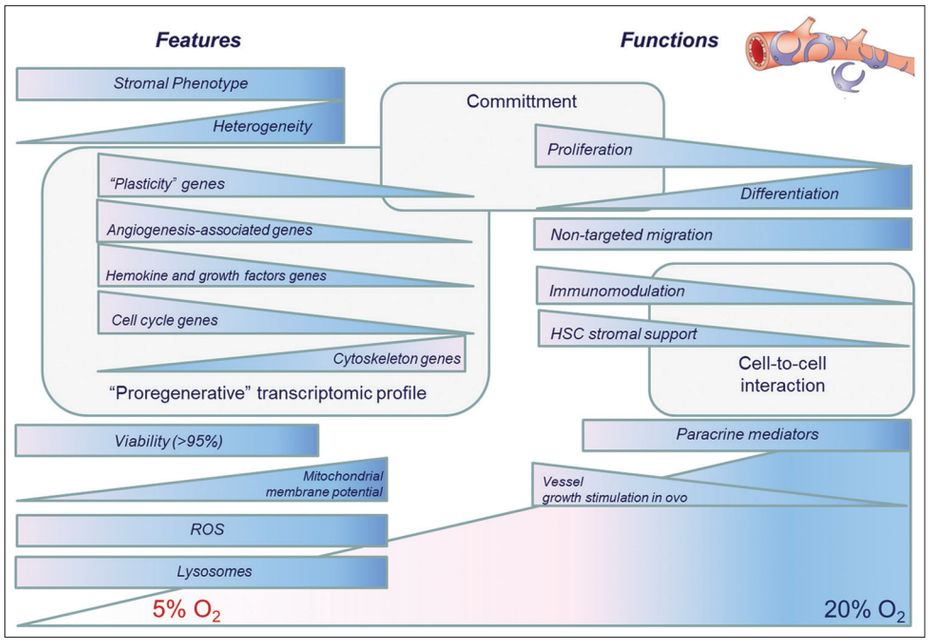

Figure 2: Modulation of ASC features and functions under "physiological" hypoxia *ex vivo*. Permanent expansion under "physiological" hypoxia does not exert damaging effects on ASCs from adipose stromal vascular fraction and is accompanied by a significant changes in their features and functions. ASCs under "physiological" hypoxia retain stromal phenotype, display homogenous fibroblast-like morphology, attenuated transmembrane mitochondrial potential. Their transcriptomic profile demonstrates "pro-regenerative" switch. ASCs exhibit enhanced proliferative activity and attenuated osteo- and adipo-differentiation. Immunosuppressive, stromal, and angiogenic potentials are potentiated at "physiological" hypoxia.

provided the increase of $CD34^+$ cells in the HSPC population and supported the preferential development of certain hematopoietic lineages.[10,11] At least a part of these effects may be connected with modulation of ASC functional activity. Under "physiological" hypoxia, ASC genes encoded proteins responsible for cell adhesion; production, and remodeling of extracellular matrix were upregulated. ASC-associated HSCs included a subpopulation of $CD45^+/CD90^+$ non-committed hematopoietic precursors, which makes them a demanded product in cell therapy and regenerative medicine [Figure 6]. In ASCs associated with HSCs, the immunosuppressive genes were upregulated (*IDO, LIF,* and *PTGS2*). These cells effectively inhibit activation and proliferation of stimulated allogeneic T cells. Transcription and secretion of the major angiogenic mediator, vascular endothelial cell growth factor (VEGF), were also increased, indicating a potential to stimulate growth of vessels.[12]

So far, a combination of the ASCs and tissue O_2 level provides pronounced stromal effects with predominant support of uncommitted HSCs (long-term culture initiating cells, LTC-ICs) that may supply long-term maintenance of hematopoiesis *in vivo*. At the same time, ASCs retain their own regenerative potential, displaying the "third tissue" properties.

Thus, *ex vivo* modeling of the MSC and HSPC tissue microenvironment makes it possible to investigate the basic mechanisms of interactions between stromal and hematopoietic cells, which can be further used to optimize the expansion protocols of committed and undifferentiated hematopoietic precursors.

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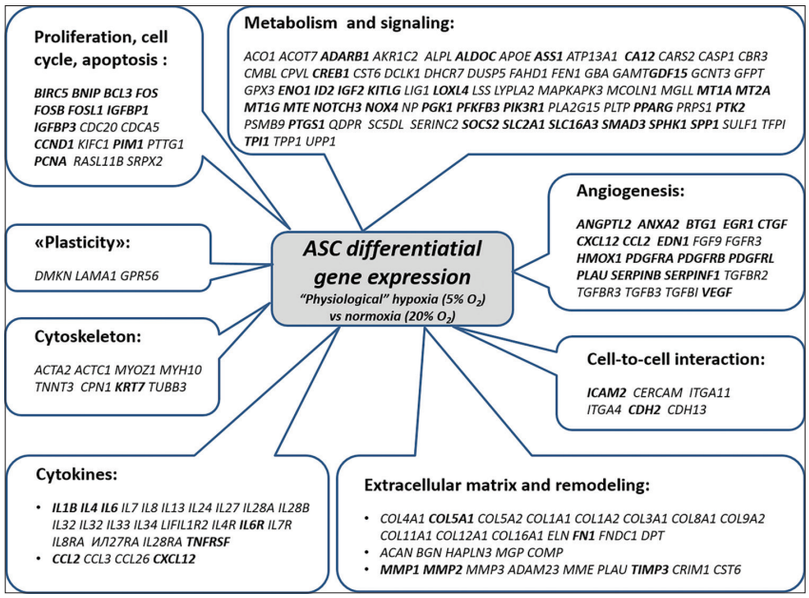

Figure 3: Microarray analysis of differential gene expression in ASCs permanently expanded under 5% and 20% O_2. The data were ranked according to gene ontology (GO) and the groups with a significant number of differentially expressed genes are presented. Genes encoded proteins with confirmed hypoxia-dependent regulation are marked bold (hypoxiaDB.com).

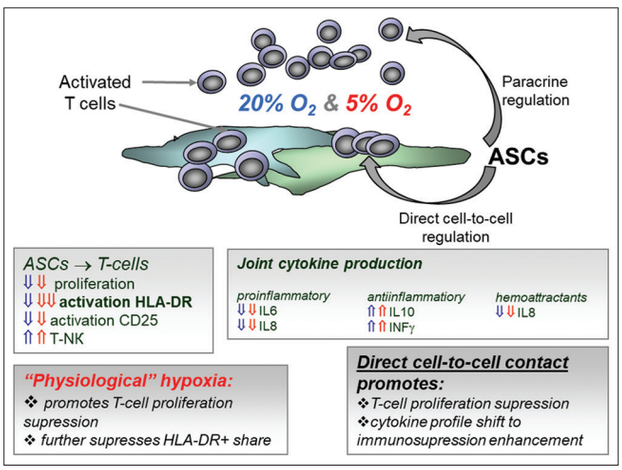

Figure 4: ASC immunosuppressive activity. Allogeneic ASCs effectively inhibits stimulated T cells at different O_2 levels. Under 5% O_2, immunosuppressive effects can be further potentiated, which may contribute to more significant reduction in the immune response by suppressing the activation (HLA-DR) and proliferative activity of T cells and shifting the balance of soluble mediators toward anti-inflammatory effects. The absence of direct intercellular contacts does not cancel, but weakens the immunomodulatory effect of ASCs.

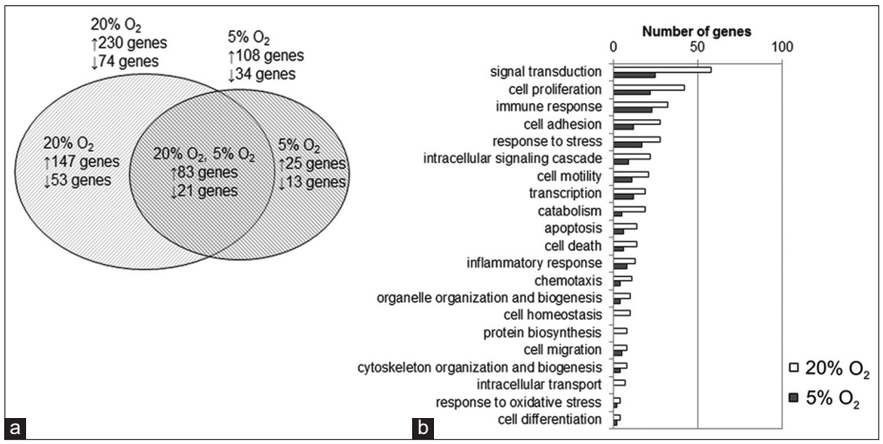

Figure 5: Characterization of differential gene expression in ASCs after paracrine interaction with PHA-stimulated allogeneic PBMCs. (a) Venn diagram, showing the number of ASC genes commonly or differentially expressed at 20% and 5% O_2. (b) The number of differentially expressed genes in gene ontology (GO) groups (biological function). Adapted from Bobyleva *et al.*[8]

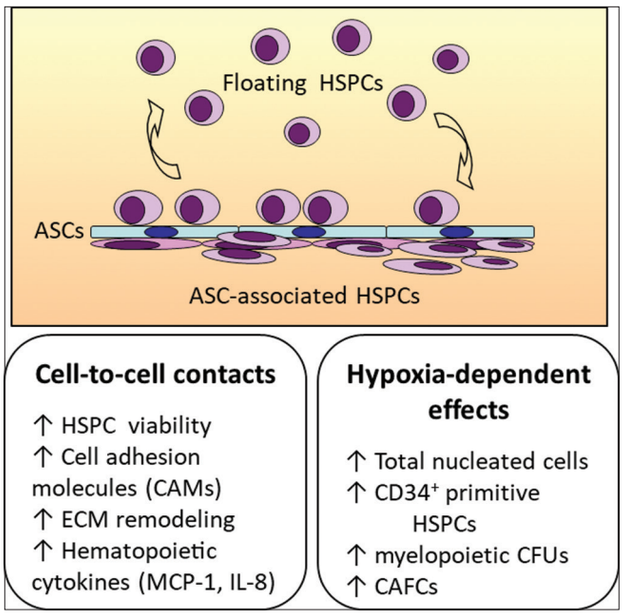

Figure 6: Interaction of ASCs and hematopoietic stem and progenitor cells (HSPCs) in coculture. To enrich the population of HSPCs, MNCs from cord blood were seeded on ASC layer and cocultured for 72 h. Then, all floating MNCs were washed out. The remaining ASC-associated population are known to be mostly composed of HSPCs. ASCs with attached cbHSPCs were further expanded at 20% and 5% O_2. During coculture, two HSPC populations were generated: the newly raised floating HSPCs and the ASC-HSPC associates. ECM: Extracellular matrix, CFU: Colony-forming unit, CAFC: Cobblestone area forming cell.

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Despite a significant progress in identifying of MSC tissue depots and a growing understanding of their role as a key players of physiological and reparative remodeling, these cells remain ones of the most "misterious" because of the lack of experimental approaches for analyzing their functional activity *in vivo*. Todays, it is already obvious that in the tissue microenvironment, MSCs not only execute supportive function but also are a source of various biologically active molecules that govern tissue niches. Due to their role of tissue homeostasis and auto-/paracrine regulation, MSCs are especially interesting from the point of view of cell senescence. It should be noted, for example, that the replicative senescence of MSCs results in a significant modification of the profiles of secreted factors involved in angiogenesis.[13]

In the damaged areas, MSCs are most likely to play the protective and probably nursing role. It could be occurred through the formation of a regenerative microenvironment, primarily due to paracrine mediators that inhibit apoptosis and scar formation, activate angiogenesis, and stimulate resident cells to restore their functional state. Both in local tissue depots and in damaged tissues, the microenvironmental factors play an important role in the realization of MSC functions. Low O_2 tension in MSC physiological niches can provide the maintenance of their undifferentiated state, which grant their urgent response, for example, accelerated proliferation during cell mobilization from the depot. On the other hand, ischemia in a damaged area triggers a cascade of molecular events that induce migration and provide MSC homing into the target tissue. In addition, low O_2 levels can apparently enhance the reparative potential of MSCs, providing effective implementation of the regenerative program.

The data from *in vitro* experiments remain the main channel for expanding our knowledge about the potential of MSCs. It would be incorrectly to expect that the MSC behavior *in vitro* is consistent with their activity *in vivo*. It is more reasonable to assume that the *in vitro* conditions enable cells to demonstrate their latent potential. That is why experimental simulation of the physiological microenvironment is of utmost importance. This assumption is fully supported by the data, according to which permanent cultivation at "physiological" hypoxia has no damaging effects on MSCs, and also results in their modifications manifested in a low committed state and enhancement of immunosuppressive potential. The above data are on applied demand for the needs of cell therapy and regenerative medicine (Russian Federation Patents N 2525143; N 2628092).

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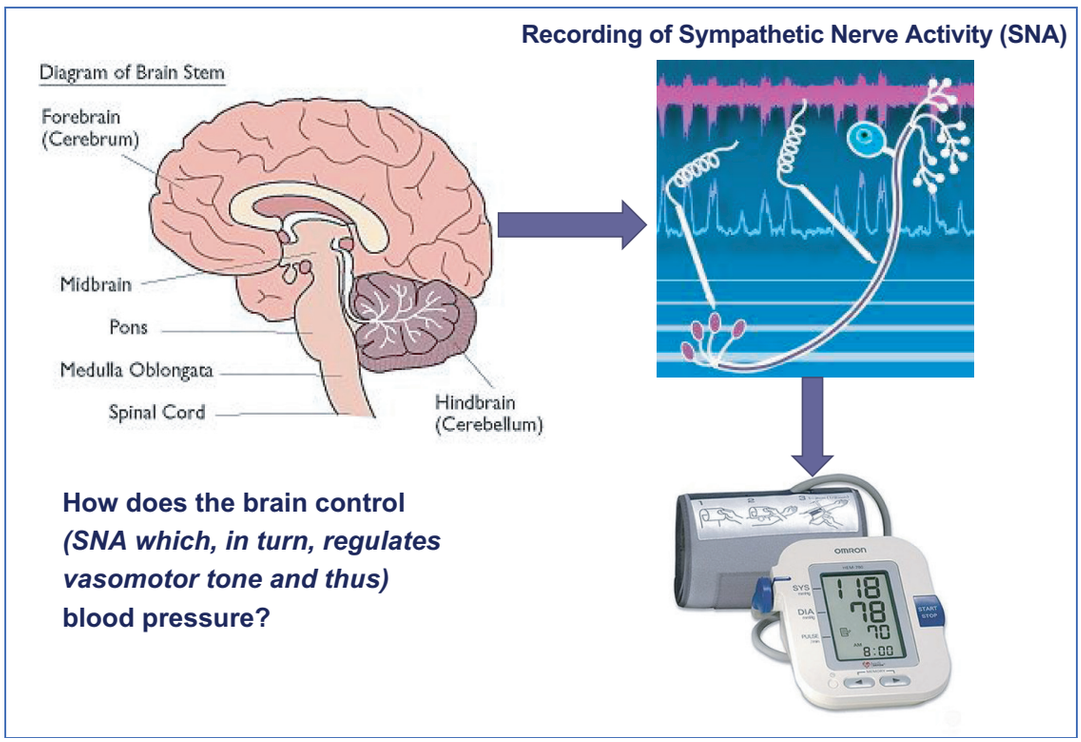
The Never-Ending Question



Courtesy: Susan M. Barman, Michigan State University, Michigan, USA.

A 2021 Status Report of Research on the Sympathetic Neural Control of the Cardiovascular System

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"In an era when the importance of integrative systems physiology is re-emerging into the spotlight of biomedical science, the sympathetic nervous system can be viewed as the ultimate integrator of systems physiology in control of cardiovascular function…Indeed, one of the most exciting aspects of measuring sympathetic neural activity is the ability of the investigators to see integrative physiology "in action" every time they do an experiment." This quote from Wallin and Charkoudian[1] articulates the significance of the sympathetic division of the autonomic nervous system in integrative physiology. Although these authors were referring specifically to the control of the cardiovascular system, the statement can relate to sympathetic-mediated control in general. Long before the technology to record sympathetic nerve activity (SNA) had been developed, Cannon[2] postulated that the sympathetic nervous system plays a vital role in the maintenance of homeostasis, allowing the organism to adapt to challenges imposed by internal and external forces. Changes in SNA have been linked to various pathologies.[3-5] For example, sympathetic hyperactivity is associated with disturbances such as neurogenic hypertension, metabolic abnormalities, cardiac dysrhythmias, psychogenic heart disease, hyperhidrosis, and thermoregulatory disturbances. Sympathetic failure is associated with dysfunctions including orthostatic hypotension, male ejaculatory failure, Horner's syndrome, and anhidrosis.

A common feature of recordings from multifiber recordings from sympathetic nerves targeting cardiovascular organs of both animals and humans is that that bursts of activity are synchronized to the cardiac cycle due to baroreceptor-induced entrainment, and the amplitude of these cardiac-related bursts varies on the time scale of the respiratory cycle due to central and reflex-induced cardiorespiratory integration.[6-8] Once the inputs responsible for the cardiac-related and respiratory-related rhythms in SNA have been removed, the synchronization of sympathetic bursts to the cardiac and respiratory cycles disappear and are replaced by other burst patterns that reflect the intrinsic ability of the central nervous system to generate different patterns of SNA. This has been studied extensively in cats in which, following bilateral section of the carotid sinus, aortic depressor, and vagus nerves (baroreceptor denervation), SNA develops irregular 2–6 Hz activity under barbiturate anesthesia and a prominent 10-Hz rhythm under urethane anesthesia.[6-8] These periodicities in SNA reflect the activity of an integrated central sympathetic neural network that include neurons in the caudal medullary raphe, caudal ventrolateral medulla, caudal ventrolateral pons, medullary lateral tegmental field, rostral dorsolateral pons, and rostral ventrolateral medulla (RVLM).[8,9] These rhythms are conveyed to sympathetic preganglionic neurons through neurons in several regions including the RVLM, caudal medullary raphe nuclei, and caudal ventrolateral pons.[7,8] The characteristics of SNA are altered during stress and particular behaviors such as the defense response and exercise, which supports the view that supratentorial regions (e.g., insular and prefrontal cortices, amygdala, and hypothalamus) contribute to the changes in SNA expressed during these behaviors.[9] Figure 1 shows a model of some key information known about the interconnections of

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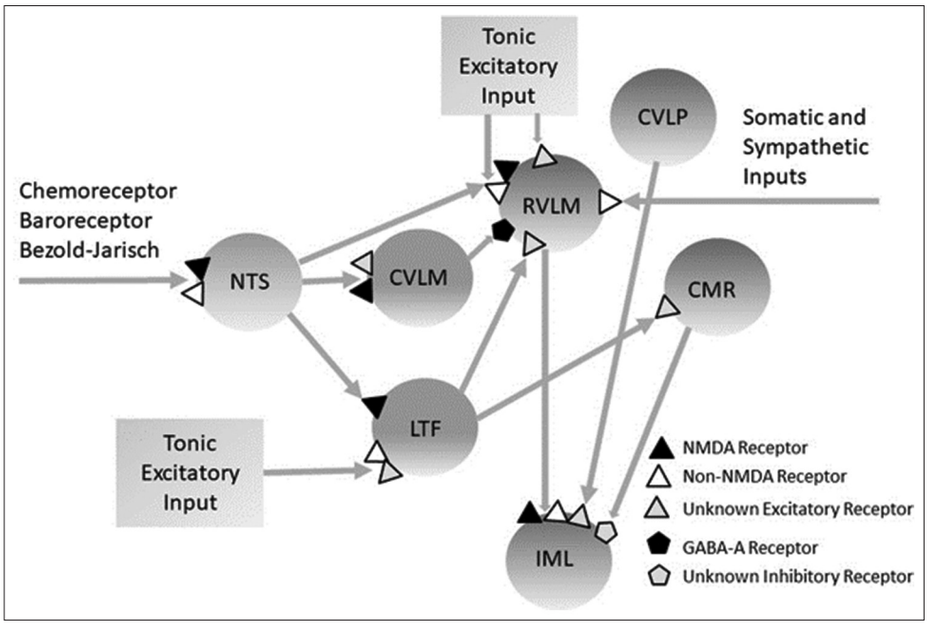

Figure 1: Central sympathetic pathways based on studies that used feline and rodent models. The basis for the various connections and receptor types are described in several recent reviews (1-3, 5). CMR: Caudal medullary raphe, CVLP: Caudal ventrolateral medulla/caudal ventrolateral pons, LTF: Lateral tegmental field, NMDA: n-methyl-D-aspartate, RVLM: Rostral ventrolateral medulla. Tonic excitatory inputs include the insular cortex, hypothalamus, and periaqueductal gray and play a role in mediating behavioral changes in SNA.

various brainstem neurons and peripheral afferents involved in the regulation of SNA. Details of these connections can be found in reviews by Barman.[6-9]

Importantly, there is evidence available that changes in the pattern (rhythmicity) of SNA without a change in the total power (magnitude) of SNA can result from some pharmacological perturbations of brainstem pathways.[6,7] The use of frequency-domain analysis revealed that blockade of N-methyl-D-aspartate receptors (a type of excitatory amino acid receptor) within the medullary lateral tegmental field of an anesthetized cat nearly eliminated the respiratory- and cardiac-related rhythms in SNA without changing the total power (total amount) of SNA. These data highlight the importance of assessing the frequency characteristics of SNA when studying the responses to various perturbations to the central or peripheral nervous system.

For the past nearly 50 or more years, many autonomic neuroscientists have relied primarily on the use of anesthetized or decerebrate animals to answer probing questions and to provide valuable insight on the organization of central sympathetic networks responsible for basal and reflex-induced changes in SNA and arterial pressure. In the 21st century, there has been considerable interest in assessing the neural control of autonomic regulation in conscious animals under conditions of both health and disease. Indeed, to gain a full understanding of how SNA is modified during affective responses, specific behaviors, and disease development and progression requires simultaneous recordings of SNA, the activity of central sympathetic neurons, and cardiovascular indices such as arterial pressure in awake, behaving animals. The results of one of the few studies to characterize the activity of presumed RVLM sympathetic neurons in conscious cats suggested that the expression of cardiac-related activity by central neurons is

labile and dependent on the animal's cognitive state.[10] This study did not include a recording of SNA or arterial pressure, so we were unable to assess whether this liability of cardiac-related activity in the RVLM was reflected by changes in resting levels or patterns of SNA and arterial pressure.

Use of state-of-the art technologies like telemetric methods for chronic recordings of SNA and arterial pressure in conscious animals has allowed investigators to determine if a change in SNA is temporally correlated to the development and/or progression of a cardiovascular disease.[11] Wehrwein and Barman[12] reviewed several publications that included recordings of SNA over the time course of development and progression of hypertension in different animal models. Perhaps surprisingly, there is a paucity of data to show explicitly that an increase in SNA precedes the onset of elevated arterial pressure. These reports did not consider the possibility that hypertension is secondary to a change in the rhythmic pattern of SNA rather than simply an increase in the level of SNA. Future studies should include an assessment of the pattern of SNA when studying the development of cardiovascular diseases.

New technologies such as optogenetics and pharmacogenetics coupled with recordings of SNA and arterial pressure could also prove to be of value in characterizing the cardiovascular function of central neurons with known neuronal phenotypes during physiological and pathophysiological behaviors.[13,14] Neurophysiological experiments in awake, behaving animal models are needed to delineate the neural mechanisms that contribute to adjusting SNA during stress and emotional states, and produce feed forward and anticipatory cardiovascular responses that occur during movement and specific behaviors.

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Nitric Oxide Signaling in the Control of Blood Pressure: The Good, the Bad, and the Ugly

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Manifests of body functions represent the outcomes of events that are integrated at multiple levels of systems, organs, tissues, cells, and molecules. When these multilevel integrations are executed in "good" rapport, our body functions are operated in the "physiological" zone. "Pathophysiological" conditions will be instigated when they turn into "bad" relationships, leading to disease development. The "ugly" scenario will emerge on break down of the multilevel integration system, which prompts "pathological" states that heads for mortality.

Control of Blood Pressure Engages Multilevel Integration

One illustrative example of the good, bad, and ugly aspects of the multilevel integration system in the operation of body functions is the control of blood pressure (BP). Maintenance of a stable BP requires integration at the level of systems (neural, hormonal, humoral, and immune systems); organs (heart, blood vessel, kidney, and brain); cells (endothelial cells, smooth muscle cells, neurons, immune cells and perivescular adipocytes); and an array of molecules, including at least nitric oxide (NO), angiotensin II, endothelin, vasopressin, and superoxide of the reactive oxygen species (ROS). This short essay shall highlight the good, bad, and ugly roles of NO in the integrative system for the control of BP. It is not our intention to provide a detailed scientific review, but to highlight the progress made in the research field on this topic.

NO the Molecule

The gaseous molecule NO has captured the spotlight of contemporary research in biomedicine when Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad were jointly awarded the Nobel Prize in Physiology or Medicine in 1998 for their discoveries concerning "nitric oxide as a signaling molecule in the cardiovascular system." NO is synthesized by NO synthase (NOS), which uses NADPH and oxygen to convert a guanidino nitrogen of L-arginine to yield citrulline as a product along with NO.[1] Four NOS isoforms have been identified in mammalian cells, namely, neuronal NOS (nNOS or NOSI), inducible NOS (iNOS and NOSII), endothelial NOS (eNOS or NOSIII), and mitochondrial NOS (mtNOS). In general, both nNOS and eNOS are constitutively expressed primarily in neurons and endothelial cells, respectively, and are responsible for basal NO release in a calcium-calmodulin dependent manner; whereas iNOS is an inducible form firstly identified in macrophage for the generation of NO in a calcium independent fashion.[2] The mtNOS is a relatively new member of the NOS family and was identified as an isoform of constitutive nNOS present in the inner mitochondrial membrane.[3] NO is a highly lipophilic molecule that has the ability of freely diffusing towards membranes to exert its biological action away from its site of formation.

NO as a "good" Molecule

The overall evidence that emerged from work published during the past three decades suggests that NO released under physiological condition is considered to be a "good" molecule. It is in essence engaged in both tonic and reflex control of BP homeostasis, as well as cardiovascular adaptations under conditions such as physical exercise and emotional stress.

The most recognizable action of NO, which instigates clinical translation of the molecule, is that on generated from eNOS in

the endothelial cells, NO diffuses into the underlying vascular smooth muscle cells where it causes elevation of cyclic guanosine monophosphate (cGMP) through the activation of cytosolic guanylate cyclase.[4] Both the cGMP-dependent protein kinases in the activation of calcium-dependent potassium channels and phosphorylation of myosin in vascular smooth muscle cells are the two salient events responsible for the well-known NO-induced vasorelaxation.[4] These ground-breaking findings also led to the realization at the end of last century that under physiological condition continuous basal release of eNOS-derived NO maintained by neurotransmitter such as acethylcholine and humoral factor such as bradykinin is responsible for the vasodilator tone of vessels;[5] whereas the release of norepinephrine by the adrenergic system maintains a vasoconstrictor tone. Moreover, it is through the dynamic balance between the neural and humoral systems that BP homeostasis is maintained under physiological condition. Endothelial NO is also vasoprotective. NO released toward the vascular lumen is a potent inhibitor of platelet aggregation and adhesion to the vascular wall.[6] It also prevents vascular smooth muscle proliferation by inhibiting the release of platelet-derived growth factors.

Soon after the characterization of the basic biological actions of NO in the vasculature, evidence of NO as a physiological mediator in other tissues and organs has emerged. A series of articles published at the end of last century and the first decade of the present millennium revealed NO as a key neuromodulator within the central nervous system, particularly in autonomic regions involved in neural and neurohormonal control of circulation, including nucleus tractus solitarii (NTS), rostral and caudal ventrolateral medulla (CVLM and RVLM), and hypothalamic paraventricular nucleus (PVN). For example, eNOS-derived NO in the PVN exerts tonic inhibition on sympathoexcitatory outflow,[7] and NO derived from eNOS in the NTS exerts a tonic inhibition on baroreflex feedback control of BP.[8] Moreover, simultaneous sympathoexcitatory and sympathoinhibitory effects of the eNOS-derived NO within the RVLM and CVLM constitute a part of physiological adaptations in central control of circulation during muscle contraction and static exercise.[9]

Both nNOS and iNOS within the central nervous system are also engaged in neural control of BP under physiological condition. Contemporary literature supports an excitatory role for nNOS-generated NO at the PVN in regulating sympathetic vasomotor outflow,[10] and modulating baroreflex sensitivity in the NTS.[11] iNOS, as the name denotes, is originally thought to be activated in macrophages, astrocytes, and microglia by immunological or inflammatory stimuli. There is now evidence that iNOS is also expressed constitutively in neurons and microglia. In the RVLM, NO derived from nNOS causes sympathoexcitation through activation of glutamatergic neurotransmission; whereas iNOS-derived NO promotes sympathoinhibition through stimulation of GABAergic neurotransmission.[10,12] Moreover, the activity of nNOS is more prevalent than iNOS under physiological conditions, such that NO-related sympathetic excitation predominates and contributes to the maintenance of basal sympathetic outflow and vasomotor tone. The RVLM neurons are also engaged in baroreflex regulation of sympathetic nerve activity. In this regard, nNOS in the RVLM contributes to the processing of the cardiac sympathoexcitatory reflexes[13] and facilitate sympathetic baroreflex transmission.[14] In addition, iNOS within the RVLM plays an active role in modulating sympathoexcitation in exercise pressor reflex.[15]

NO can also be produced by mtNOS locally in the mitochondria.[16] Excessive intra-mitochondrial NO production inhibits the activity of Complexes I and IV in the mitochondrial electron transport chain to yield mitochondrial ROS production.[17]

NO as a "bad" Molecule

Given its indispensable role in the maintenance of BP homeostasis, a reduction in tissue NO bioavailability would in theory lead to "bad" consequences of cardiovascular functions. Indeed, one interesting and definitive conclusion from over more than 120,000 scientific publications on NO during the past 30 years is that individuals with clinically diagnosed atherosclerosis, diabetes, or hypertension often show impaired NO signaling pathways. For example, hypercholesterolemia and atherosclerosis are associated with impairment of endothelium-mediated vasodilatation in the vasculature, mainly due to increased circulating levels of the endogenous NOS inhibitor asymmetric dimethyl-L-arginine.[18] A blunted vasodilatation in response to acetylcholine has also been documented in both animal models of and patients with hypertension. A variable number of tandem repeats in intron 4[19] and a mis-sense variant, Glu298Asp, in exon 7[20] of the

eNOS gene were found to be significantly associated with human essential hypertension. Later work in animal studies demonstrated that endothelial NO deficiency may result in an abnormal vascular phenotype and instigate pathological changes in the vessel wall associated with hypertension[21] and atherosclerosis.[22]

A literature survey further suggests that NO in the brain exhibits both neuroprotective and neurotoxic effects on central circulatory control that is dependent on the pathophysiological stages. For example, at the early stages of cerebral ischemia, a surge in NO release generated by eNOS seems to protect neurons from death by inducing vasodilatation and inhibiting microvascular aggregation.[23] However, overproduction of NO by nNOS or iNOS in later stages of stroke contributes to apoptosis and subsequent neuronal death.[5] Activation of iNOS precipitates decrease in BP following cytokine release induced by the endotoxin, *E. coli* lipopolysaccharide.[24]

Further investigation revealed that the various biological actions of NO are likely dependent on the gas production kinetics of the different NOS isoforms. The constitutive NOS (i.e., nNOS and eNOS) produces pulsatile release of a very small (in nM range) amounts of NO; whereas iNOS is responsible for the generation of a larger amount of NO (in μM range) release over longer periods.[25] As such, NO that plays a physiological role in BP regulation when it is produced by the constitutive NOS could become a pathophysiological entity when generated by iNOS. Although the exact amount of NO produced *in situ* by each of the NOS isoform under physiological or pathophysiological state cannot be accurately quantified, evidence shows that when activated, one iNOS molecule can generate several hundred to several thousand times more NO than one nNOS molecule.[26] This excessive amount of NO released in tissues could well be responsible for the "bad" face of the molecule in the control of BP.

NO as an "ugly" Molecule

The "redox nature" and cytotoxic actions of NO did not attract much attention from the physiology community when Blough and Zafiriou[27] made the original demonstration that showed that NO can react with superoxide (a member of ROS family) in aqueous solution to yield peroxynitrite anion, a potent biological oxidant, and reactive nitrogen species.

In fact, this reaction was initially taken by the physiological community as a way to "regulate" the biological half-life of NO; a reaction perceived as an "oxidative inactivation" of NO for its degradation to yield unreactive products, mainly nitrate and nitrite. It was the much later discovery of peroxynitrite as a biologically relevant cytotoxic intermediate[28] that spurred research on the "ugly" side of NO.

In the context of BP control, the markers of peroxynitrite generation have not only been documented in animal models of shock but also in human specimens obtained from patients suffering from circulatory shock.[29,30] There is a significant correlation between the degree of nitrotyrosine formation and the severity of the clinical condition in human sepsis. We now know that peroxynitrite, primarily generated through the reaction of the iNOS-induced NO and superoxide, plays a significant pathogenetic role at least in vascular hyporeactivity, capillary extravasation, tissue edema, myocardial hypocontractility, pulmonary, and renal injury associated with circulatory shock.[31] Under a pathological condition exemplified by experimental brain stem death, overproduction of NO generated by massive activation of iNOS in the RVLM, coupled with the impending augmentation of superoxide, elicits presynaptic inhibition on glutamate release through the formation of peroxynitrite, leading to prolonged sympathoinhibition that results in severe hypotension.[32,33]

Concluding Remarks

For the sake of simplicity and convenience, it is not uncommon for physiologists to label NO as a "good," "bad" or "ugly" molecule. As pointed out in this brief review and illustrated by control of BP, this is conceptually incorrect. Whether NO acts as a "good" or "bad" signaling molecule or an "ugly" cytotoxic agent largely depends on its concentration and the redox environment at the site where its actions take place, and are contingent on it engagements in a physiological, pathophysiological, or pathological process.

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Students and staff of the Department of Physiology, Lagos State University, Nigeria performing nucleic acid extraction in the biosafety cabinet. Courtesy: Simiat O. Elias, Lagos State University, Lagos, Nigeria.

Salt Sensitivity and Other Determinants of Blood Pressure

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"*When salt was not readily available, it was a relatively essential commodity, but in the modern world salt has become plentiful, and it is actually difficult to achieve a low salt intake without exerting a significant amount of effort.*"[1]

Essential hypertension is high blood pressure without any discernible cause; it represents over 90% of all diagnosed hypertension cases. Recently, it has been subdivided into salt sensitive and salt resistant hypertension. Salt sensitivity is the propensity to develop great increases in blood pressure to increased dietary sodium intake. It is not a homogeneous definition as some people found to be initially salt resistant may become salt sensitive with age. Kawasaki *et al.*[2] first described this phenomenon by subjecting a group of health volunteers to different dietary salt content: a "normal" (109 mmol/d), "low" (9 mmol/d), and "high" (249 mmol/d) sodium-containing meals. A specific diagnostic technique for determining salt sensitivity has eluded researchers over the years. According to the American Heart Association, "there is no evidence base to determine best research practices in terms of measurement of salt sensitivity of BP in humans."[3] The gold standard for testing for salt sensitivity is a protocol without the inclusion of a diuretic as shown in Table 1, modified from Kurtz *et al.*[4] with source information included[5-7] In our lab, we had followed the method of Weinberger *et al.*[8] in which salt sensitivity was determined as an increase of ≥5 mmHg in mean arterial pressure (MAP) following the salt load, while those with a difference of <5 mmHg were considered salt-resistant. We recorded 55.8% and 34.0% salt sensitivity among the hypertensive (HT) and normotensive (NT) participants, respectively.[9]

Salt sensitivity is not only associated with increased incidence of hypertension but also even NT salt sensitive individuals may develop target organ damage in the heart, kidneys, and blood vessels. Furthermore, salt sensitivity generally leads to early mortality, whether the individual is HT or not.[10]

It is unclear what mechanisms determine who becomes salt sensitive, although we know that the kidney, blood vessels, cardiovascular system, and the autonomic nervous system are important. The kidneys are thought to be involved by retaining large amounts of salt with the associated volume of water leading to overloading of the vascular system; this leads to an increase in cardiac output thereby elevating blood pressure. More recently though, salt is thought to increase cardiac output which then leads to high blood pressure; this would not be sustained under normal circumstances because of the vasodilation that typically follows. But in salt sensitive persons, especially those of African origin, there is inhibition of this vasodilation therefore leading to hypertension.[11]

Salt taste sensitivity is the capacity to identify the flavor of salt and as this influences one's salt-intake, it also influences the risk for a salt-sensitive person to develop hypertension. The World Health Organization has recommended the daily intake of salt to be <5 g NaCl (<87 mmol) to prevent chronic diseases; however, people especially in most industrialized societies, ingest well above this limit.[12,13] Salt is used as a preservative for a lot of food including biscuits, bread, cereal, and fast foods like pizza. We studied the salt taste threshold and the osmoreceptor response to an acute water load (AWL) in young healthy volunteers. The participants were grouped into low salt taste threshold (LSTT; 24%) and high salt-taste threshold (HSTT; 76%) with the cutoff point at 60 mmol/L.[14] Diastolic blood pressure (DBP) increased significantly ($P < 0.02$) among the HSTT while heart rate increased significantly ($P < 0.002$) among the LSTT following the AWL. Plasma osmolality (POsm) is a tightly-regulated parameter in humans. The osmoreceptor response involves an integration of thirst, arginine vasopressin (AVP) and the renal response to AVP.[15] Following the AWL, POsm decreased significantly ($P = 0.0011$) among the LSTT, and there was

Table 1: Candidate reference method of testing for salt sensitivity.

Dietary protocol with the following features
1-week period of low salt intake of no more than 50 mmol NaCl/day
1-week period of high salt intake of 250 mmol NaCl/day*
Order of administration of different salt diets may vary per study objective
Prescription and monitoring of well-characterized diets throughout entire study†
Multiple measurements of 24 h urine Na+ excretion to confirm NaCl intake
BP measurements based on a highly reproducible salt sensitivity test protocol‡
Cutoff to classify normotensives as salt sensitive: MAP change=3–5 mmHg§
Cutoff to classify hypertensives as salt sensitive: MAP change=8–10 mmHg§

Modified from.[4] BP: Blood pressure, MAP: Mean arterial pressure. *For double-blind, placebo-controlled testing of the BP effects of changes in salt intake, the high salt intake and the placebo can be administered in unmarked capsules. †Because potassium,[5] nitrate,[6] and other dietary factors can affect BP, the contents of the diets should be carefully described for each study phase and contents should not be varied unless required as part of the study objective. Based on the diets that were used in studies of protocols with demonstrated high reproducibility for classifying subjects as salt sensitive,[7] a dietary potassium intake in the range of 60 to 80 mmol/day could be recommended. ‡Details of BP measurement techniques and the BP cutoffs used in test protocols reported to be highly reproducible can be found in Draaijer *et al.*[7] §The cutoffs in these ranges should be pre-specified. If the high salt intake amount happens to be somewhat lower than the target salt intake of 250 mmol/day, the cutoff may be based on the lower number in the recommended cut-off range. If the amount of salt administered is very close to, or somewhat above, the target salt intake of 250 mmol/day, the cutoff may be based on the higher number in the recommended cut-off range

a slight increase in plasma AVP among both groups. These results led us to conclude that salt-sensitive subjects (HSTT) elicit a greater response to an AWL than salt-resistant subjects (LSTT).

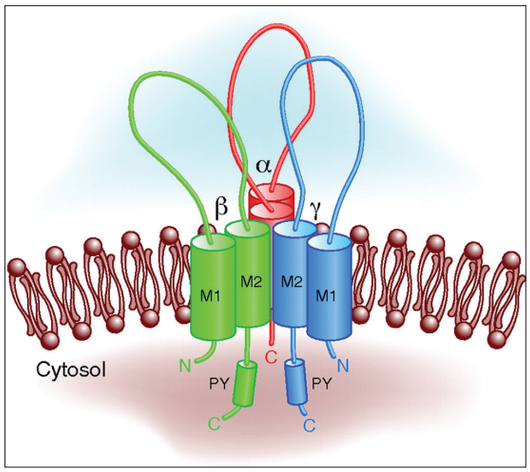

Figure 1: Structural features of the epithelial sodium channel. Modified from.[18]

Salt Sensitivity and the Epithelial Sodium Channel (ENaC)

There has been a flurry of activity geared toward understanding the role played by genetics in determining salt sensitive hypertension. Hypertension is inherited in the Mendelian fashion. Genetic diseases with Mendelian transmission are often characterized by mutations with rare allelic frequency in human populations (<0.1%). On the other hand, allelic polymorphisms are more frequent and have an allelic frequency of >1% or 2% in human populations.[16] These allelic polymorphisms are usually single nucleotide polymorphisms but others such as copy number variations or short tandem repeats are also possibilities.[16] The combined action of many genes as opposed to a single gene may determine the blood pressure response to salt ingestion. Each of the genes may affect one or more channel, transporter, or enzymes associated with neural, hormonal, vascular and renal control mechanisms of blood pressure.[17]

The ENaC (with three structurally-related subunits α-, β-, γ- subunits, shown in Figure 1[18]) is only one of the genes that have been associated with human essential hypertension.

This channel plays a critical role in the control of sodium balance, blood volume, and blood pressure.[19] We reported the presence of polymorphisms of the β-subunit of ENaC among NT and HT volunteers.[20] Five percent of the subjects had the β-T594M polymorphism, (HT 3/53; NT 2/47; $P = 0.75$); the two NT were also salt sensitive. We recorded four previously unreported mutations of β-ENaC: E632V and E636V, respectively, in two HT participants, D638Y in another HT, and L628Q in one NT volunteer.[20] The β-T594M polymorphism has been associated with increased blood pressure in an English population of African ancestry and Ghanaians in Kumasi[21] whereas no similar association was found in a South African population. Further studies are required to establish its role in the salt sensitivity of blood pressure and development of hypertension among our populace.

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Impact of Arterial Wall Characteristics on Cardiovascular Function

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Thomas Sydenham, "A man is as old as his arteries."

The leading role of cardiovascular diseases for morbidity, impaired quality of life and mortality calls for revealing the key underlying mechanisms and establishing the appropriate primary and secondary prevention.

The idea of the aging cardiovascular continuum was proposed as an update of the classic cardiovascular continuum.[1] According to O'Rourke *et al.*,[2] the described structural changes driven simply by aging progress to end-stage cardiac, cerebral, and renal disease [Figure 1]. This process involves the pathological cross-talk between small and large arteries through the excess of pulsatile energy.[3]

Alterations in the arterial wall characteristics (arterial wall stiffening) occur as a natural sequel of aging. This process is accelerated by a number of noxious factors: Hypertension, hyperglycemia, dyslipidemia, oxidative stress, genetic predisposition, etc. Typical is the irreversible changes in the elastin fibers leading to fractures of the elastic lamellae in the arterial media, replaced by collagen and ground substance, migration of smooth muscle cells, and impaired relationships between endothelium and vascular smooth muscle cells.[4] Eventually, thinning and dilation of the large arteries are observed that leads to grave functional consequences.

The large arteries carry out two important functions in the circulation: Conduit and cushioning. Since large arterial resistance is low, the conduit function is hardly ever impaired. The goal of the cushioning function is buffering the pressure and flow oscillations that result from the intermittent cardiac contractions. Arterial stiffening produces complex hemodynamic effects due to:

- *The increase of the "characteristic" impedance of the aorta* that accounts for the augmentation of the early systolic peak in the pressure waveform (P_1) [Figure 2]. The increase of the early systolic peak depends on the interrelation between the degree of aortic wall stiffening and the extent of its enlargement.
- *The increased pulse wave velocity* (PWV) that induces earlier return of the summated reflected pulse wave with pronounced mid-to-late systolic effect on the pressure waveform profile (earlier and augmented P_2 peak higher than P_1). Elevated aortic systolic pressure increases the left ventricular afterload. For any blood pressure level, the specific pressure waveform pattern is of relevance for the ventricular-vascular interactions.[5] Stiffening of central arteries has a number of adverse hemodynamic consequences: Increased pulse pressure, reduction of shear stress, and increased pulsatility of microcirculatory blood flow.[6-8]

The two generally applied methods for arterial stiffness evaluation are PWV measurement and arterial pulse wave analysis (PWA). PWV assessment is based on the evaluation of the time lag between the feet of two pulse waveforms from two arterial sites (proximal and distal). The golden standard for PWV evaluation is the measurement of carotid-femoral PWV (cf-PWV).[9] It is assumed to be the best option since it measures the velocity of pulse wave propagation along elastic arteries only. A large European study based on data from 1455 healthy normotensive individuals elaborated the normal PWV reference values for the different age groups.[10] cf-PWV is believed to integrate the impact of age, BP, and their interactions. A large meta-analysis based on data from 16 studies and 17 635 participants showed cf-PWV to be a reliable predictor of future cardiovascular events and a means for risk stratification.[11] At present, assessment of cf-PWV is applied mostly in scientific research and has not yet been introduced widely in clinical practice. The reasons are

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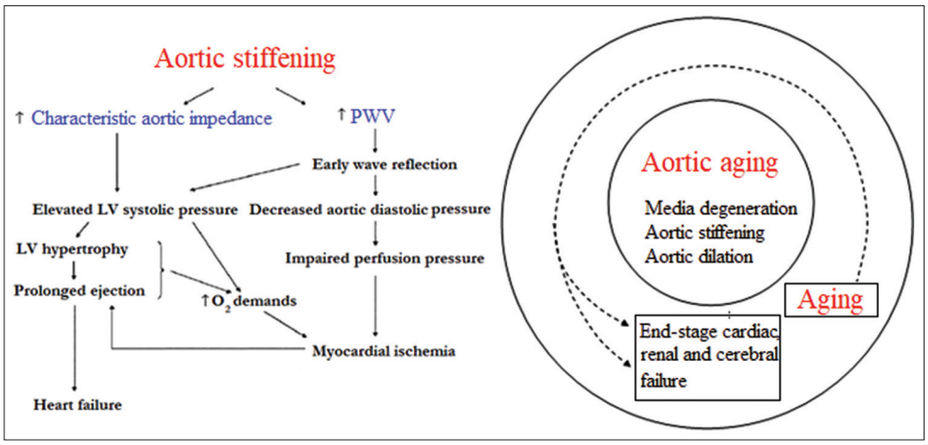

Figure 1: The model of the aging cardiovascular continuum showing the progressive alterations in the large arterial wall and its pathological consequences in cardiac function (Modified after 2).

that certain difficulties with obtaining precise carotid artery applanation exist as well as a risk of baroreceptor activation and of dislocating carotid arterial plaque.[12] Alternatively, brachial-ankle PWV (ba-PWV) has been recommended and introduced into everyday practice in Japan and other South-Eastern Asian countries.[13] A large study carried out on a total of 2916 Japanese individuals suggested that ba-PWV was a reliable predictive factor for cardiovascular disease and it improved cardiovascular risk assessment.[14] In a small pilot study carried out on 44 young normotensive individuals (22.5-years-old), we have shown ba-PWV to be significantly higher in those with family history for elevated cardiovascular risk (parents with hypertension, myocardial infarction, brain stroke, and/or diabetes) as compared to those with negative family history.[15]

Analysis of the aortic pulse waveform is also applied for arterial stiffness assessment.[16,17] Several PWA parameters are computed on the basis of radial applanation tonometry and a validated software [Figure 2].

Additional derivative indices are calculated from those parameters shown in Figure 2. Augmentation index (AIx) is the augmentation pressure presented as a percent of aortic pulse pressure $\left(\frac{AP}{PAP}\times 100\right)$. Since the timing of the reflected wave return depends on cardiac cycle duration, a corrected for standard heart rate index is recommended (AIx75%). A lot of studies evidenced AIx75 % to be a cardiovascular risk marker like PWV.[18,19] In a study carried out on clinically healthy individuals divided into two age groups (20 and 60-year-old), we examined the effect of the active orthostatic test on central hemodynamics.[20] We have shown the presence of altered cushioning arterial function in the 60 y/o group based on the significantly higher central systolic and pulse pressures, and AIx75% baseline. Furthermore, the older group responded to the orthostatic challenge with less efficient compensatory response, that is, moderate decrease of central systolic and pronounced reduction of central pulse pressure. These results were supported by heart rate variability analysis that demonstrated lesser sympathetic activation in the older individuals during upright standing. We interpreted this result as an evidence of impaired baroreflex sensitivity due to the "physiological" stiffening with aging.

An important consequence to the changes in central hemodynamics with aging is the altered vascular ventricular coupling due to the increased ventricular systolic stress and the resulting increase in myocardial oxygen demands. PWA provides an option for non-invasive estimation of these interrelationships using the tension-time index (TTI), a parameter introduced in the late 50 s of last century by Sarnoff *et al.*[21] TTI might be calculated from the area under the systolic portion of the aortic pulse waveform [systolic pressure

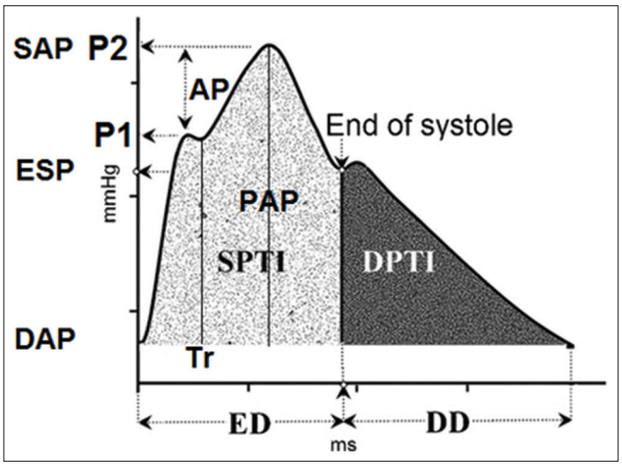

Figure 2: Central pulse waveform. SAP: Systolic arterial pressure, DAP: Diastolic arterial pressure, PAP: Pulse arterial pressure, AP: Augmentation pressure, ED: Ejection duration, DD: Diastole duration, ESP: End systolic pressure, Tr: Time to return of the reflected wave, SPTI: Systolic pressure time integral, DPTI: Diastolic pressure time integral.

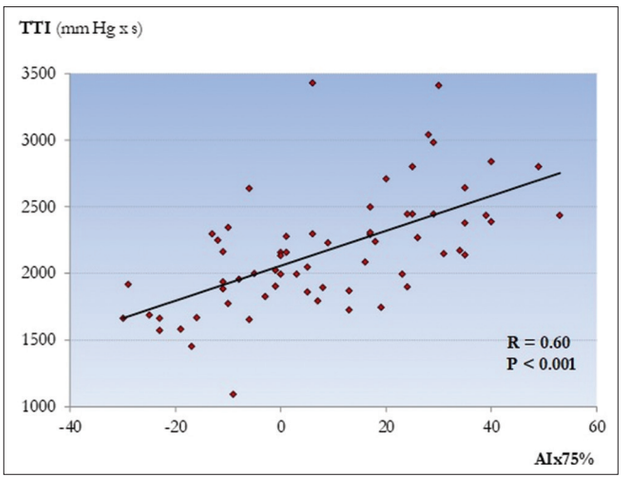

Figure 3: Positive correlation exists between AIx75% and TTI (mm Hg x s) (R = 0.60, *P* < 0.001).

time integral, Figure 2]. In a continuation of our research, we have analyzed vascular ventricular coupling in a total of 65 healthy persons divided into two groups: 50 y/o and 20 y/o.[22] TTI was significantly higher in the older group although peripheral systolic pressure in this group was in the normal range and peripheral pulse pressure did not differ significantly from the younger subjects. Moreover, we have demonstrated a significant positive correlation between the AIx as a marker of arterial stiffening and TTI as an index of myocardial load [Figure 3], we interpret this finding as an evidence in support

to the concept of the aging continuum of O'Rourke *et al.* The increased stress in the left ventricular wall during systole due to the increased pulsatile load in the systemic circulation leads to remodeling of the ventricular muscle, diastolic dysfunction, disbalance between oxygen demands and oxygen supply, and finally opens the route to the pathological spiral.[2]

In conclusion, the efforts of preventive cardiology up to now were focused on the prophylaxis of coronary atherosclerosis and endothelial dysfunction as the major causative factors of heart failure. Turning the attention of medical society to another facet of heart failure pathogenesis is important and promising. Early evaluation and active interference with vascular aging might be a potent tool in the prevention of cardiovascular pathology.[23]

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Postgraduate students – working under the mentorship of professor Faadiel Essop - pursuing exciting research work in the Centre for Cardio-metabolic Research in Africa (CARMA) at Stellenbosch University in South Africa. Pictured from left to right are current MSc students: Miss Megan Cairns, Mr. Logan Smith and Miss Hannah Geddie. Courtesy: F. Essop, Stellenbosch University, Stellenbosch, South Africa.

Doubling Down on the Dual Burden of Cardiovascular Diseases

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During the last years, our research work focused on the "dual burden" of cardiovascular diseases (CVD) as highlighted by the World Health Organization. This refers to (a) the escalating global CVD burden that is occurring largely due to lifestyle-related choices, and (b) the increasing onset of HIV-related CVD. We embarked on this path as this issue is highly relevant within the developing world context. Our research work over the last few years is summarized as follows:

Exploring Hyperglycemia-related Cardiovascular Complications

Since a significant proportion of diabetic individuals develop CVD, we set out to delineate underlying mechanisms driving this process. In particular, we hypothesized that high glucose availability activates non-oxidative glucose pathways (NOGPs), for example, the hexosamine biosynthetic pathway (HBP) – with detrimental downstream effects. We initially set out to determine whether the HBP is actually activated with diabetes and found increased *O*-GlcNAc levels (HBP terminal modification) in diabetic individuals.[1] This work elicited strong interest since it was only one of two studies (globally) reporting such changes, that is, one on red blood cells and our work on white blood cells and offers diagnostic potential. We also investigated downstream effects of hyperglycemia on heart function in response to ischemia-reperfusion with the eventual aim to test novel cardioprotective agents. We employed *ex vivo* rat perfusions and our data revealed that acute hyperglycemic exposure caused impaired cardiac function following ischemia-reperfusion. This was mediated by higher oxidative stress and coordinated NOGP induction and allowed us to assess novel therapeutic interventions. For example, when oxidative stress or the NOGPs were inhibited this resulted in improved cardiac function.[2] Keeping the developing world context in mind, we also evaluated a plant extract (oleanolic acid) and found that it offered unique protection to hyperglycemic hearts exposed to ischemia-reperfusion.[3] We next shifted our emphasis to the ubiquitin proteasome system (UPS) as a therapeutic target and found that that partial protease inhibition (PIs) triggered protective effects and we concluded that the UPS emerges as a unique therapeutic target for the treatment of ischemic heart disease under diabetic conditions.[4] Such work culminated in a major review article that helped map ways whereby NOGPs actually mediate hyperglycemia-related cardiovascular pathologies.[5] We also pointed the way ahead in terms of future research work and highlighted novel therapeutic targets.

More recently, we extended our work within the therapeutic domain and found that the clinical drug trimetazidine offered cardioprotection to mice subjected to an in-house developed *ex vivo* acute heart failure protocol.[6] We also launched studies that examined the role of sugar-sweetened beverages (SSB) on cardiovascular function and established a unique rat model of longer-term SSB intake[7] and reported dysregulation of the NOGPs as an early metabolic change in this case.

Investigating Factors Contributing to HIV-related CVD Onset

We established a novel rat model of chronic anti-retroviral (ARV) exposure by implanting mini-osmotic pumps into rats. This ensured the steady delivery of HIV PIs over an extended

period and resulted in higher body weights, increased circulating LDL levels and tissue triglycerides, metabolic gene alterations, and decreased heart function together with an impaired UPS.[8-10] Together these studies show that HIV PIs directly lowered myocardial UPS function and that this initiated transcriptional changes that contributed to perturbed lipid metabolism, thereby fueling a pro-atherogenic milieu. This was followed by studies evaluating whether a natural compound (resveratrol) could attenuate such effects – useful within the developing world context. Our data revealed that resveratrol enhanced cardiac mitochondrial respiration for rats subjected to PI treatment, offering therapeutic value.[11]

More recently, we moved our studies into the clinic within the Worcester region of South Africa where we are investigating the prevalence of cardio-metabolic risk factors in HIV-positive individuals. This is an ongoing study but recent published data showed that higher waist-to-hip ratios in females on longer-term ARVs potentially increases their risk for cardio-metabolic complications.[12] We recently showed a significant interplay between adaptive immune cell activation and monocyte/macrophage markers in especially HIV-positive individuals with virological failure and on second-line treatment.[13] These findings also demonstrate a unique link between immune activation and lipid subclass alterations, revealing important changes that can be missed by traditional lipid marker assessments (e.g., LDL and HDL). We recently completed an invited review article on the emerging role of immunometabolism within the context of HIV-related CVD onset, where several novel hypotheses were put forward that link immunometabolic changes to HIV-mediated CVD onset.[14]

Together, we propose that lifestyle-related CVD risk factors in non-infected individuals and HIV- and ARV-mediated CVD onset share common pathways [Figure 1]. Here key downstream targets include hyperglycemia-mediated oxidative stress and metabolic remodeling such as detrimental NOGP activation. This is also linked to UPS dysfunction and the establishment of a pro-inflammatory milieu. For HIV-infected individuals, ARVs may trigger similar pathways while the virus itself can also fuel this. Of note, lifestyle-related CVD risk factors are also highly prevalent in HIV-positive individuals especially within developing countries.

At present, we are continuing to focus on the "dual burden of disease" and have expanded our work to include psychosocial stress-related cardio-metabolic diseases onset. In parallel, there is a strong focus on growing the newly-established

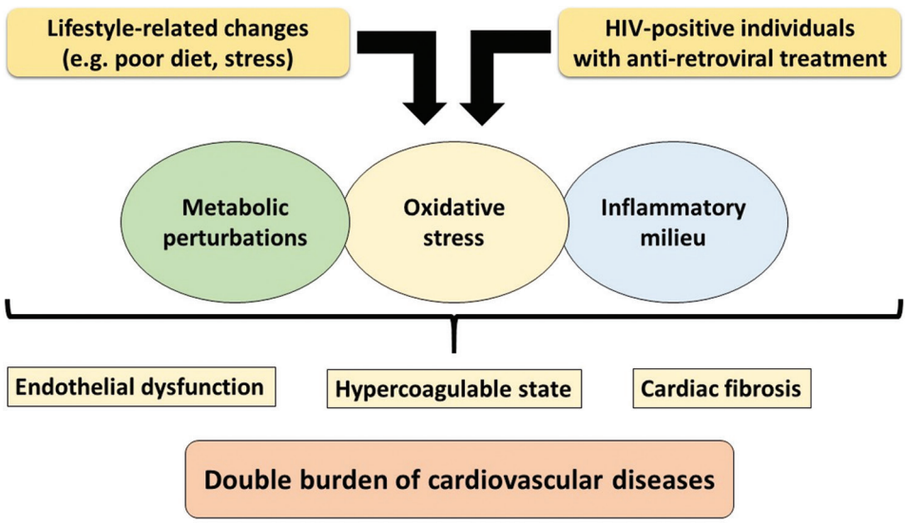

Figure 1: The double burden of cardiovascular diseases onset: Potential mechanisms.

Center for Cardio-metabolic Research in Africa (CARMA) at Stellenbosch University in South Africa. CARMA aims to function as a node of excellence to ensure integrated and enhanced research efforts with the aim to enhance knowledge creation, deliver postgraduate students and to improve health care and well-being of patients.

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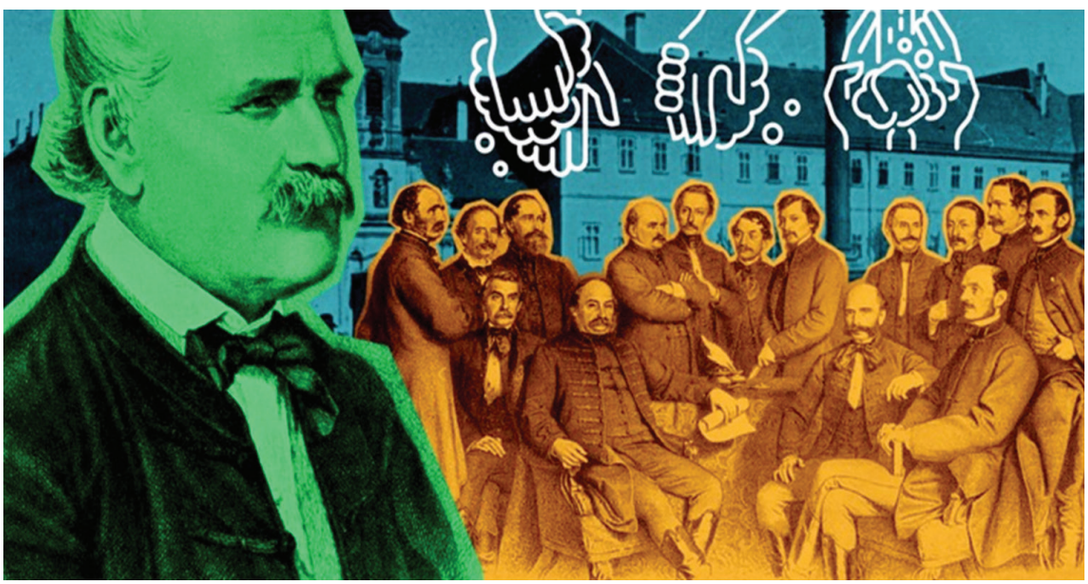

Ignaz Semmelweis (1818-1865) Hungarian physician-scientist, an early pioneer of antiseptic procedures Artist: Composition by Silmara Mansur (COC/Fiocruz) based on works by Jenö Doby, József Marastoni and György Kłösz Courtesy: Maria Jose Alves da Rocha, University of São Paulo, Brazil.

Unraveling the Aftermath Effects of Sepsis on the Brain

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For decades, physicians ignored the use of simple hygienic measures, like washing hands, in the prevention of patients' contamination, even though after Semmelweis, already in 1847, reported an incontestable link between them. Morover, it took several years until the association between bacteria and putrefaction was demonstrated by Louis Pasteur in 1863. Therefore, death due to puerperal fever that affected women, soon after delivery, and gangrene (also known as putrid fever or hospital fever) that affected wounded soldiers, were common and only later recognized as the same disease (see review by Cavaillon and Chrétien).[1] This disease nowadays would be called sepsis, a term derived from the Greek verb sepein, which means putrefy or rot.[2] We now know that the presence of pathogenic microorganisms, such as Gram-negative or Gram-positive bacteria, fungi, parasites or viruses, in tissues, fluids, or body cavities that are normally sterile, leads to infection. The infectious process triggers an inflammatory response from the host, the magnitude of which may differ in each individual. The complex interaction between the organism and the infectious agent results in the pathophysiological process that was formerly known as septicemia and today is called sepsis.[3] This disease, now recognized as a syndrome, consists of an exacerbated general inflammatory response of the organism that can cause organ dysfunction, septic shock, and in some cases lead to death.[4] If the patient survives, the consequences can be cognitive dysfunctions, such as language problems, mental and spatial confusion, and memory loss.[5] Several efforts have been made in the search for treatments for this disease, but the mortality in intensive care units continues to be high, all over the world.[4] Research groups have investigated autonomic, hormonal, and cognitive alterations following experimentally induced sepsis to understand what happens with patients who went through sepsis and survived this life-threatening process.[6-8] The cognitive and physical diabilities that can happen in patients that survive sepsis result from several physiological alterations induced by a complex immunological response during the disease. Inflammatory mediators, such as tumor necrosis factor, reactive oxygen species (ROS), and nitrogen species (RNS), such as nitric oxide released by leukocytes in the infection focus or in the blood can directly or indirectly reach the brain.[8,9] In experimental sepsis, induced by cecal ligation and puncture surgery, we can see an overproduction of ROS that can cause damage in different brain regions.[8,10] The mechanisms of cerebral oxidative stress can be associated with mitochondrial dysfunctions, redox imbalance, and a decrease in intracellular ascorbate concentration. An imbalance in the activities of the two main brain antioxidant enzymes, superoxide dismutase, and catalase, has been related to the high mortality rate seen in this experimental sepsis model and to the incidence of septic encephalopathy, a brain dysfunction that can affect up to 70% of the patients.[11] An indicative of oxidative damage is also denoted by an increase in thiobarbituric acid reactive substances and a decrease in citrate synthase activity, a marker of mitochondrial dysfunction.[8,10] As a consequence of this dysfunction, we can see cell death and neurodegeneration. The presence of apoptosis markers in septic rats is observed in brain regions that synthetize endocrine hormones like vasopressin whose secretion is impaired or altered during sepsis and persists in survival animals.[12,13] The damage is also seen in regions related to cognition, such as the cortex and hippocampus, that alters the surviving animals' behavior.[8,10]

In search for agents that could unravel the aftermath effects seen in sepsis surviving patients, clinical studies report that simvastatin, a medicament of the statins class that is ubiquitously used to decrease cholesterol levels in the blood to prevent cardiovascular diseases, could have the potential

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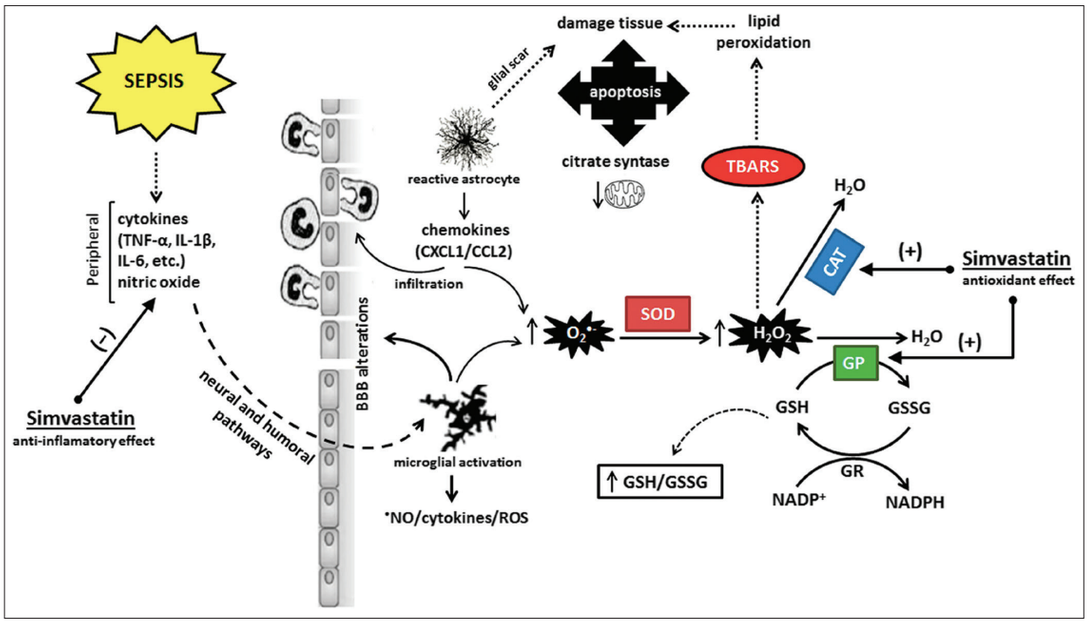

Figure 1: A scheme to explain how simvastatin treatment would affect the brain oxidative stress in sepsis. BBB: Blood brain barrier, CAT: Catalase, GP: Glutathione peroxidase, GSH: Reduced glutathione, GSSG: Oxidized glutathione, GR: Glutathione reductase, NO: Nitric oxide, ROS: Reactive oxygen species, SOD: Superoxide dismutase, More information see Molecular Neurobiology 54:7008-7018, 2018.

to protect the brain during the general inflammatory response seen in sepsis. By diminishing blood cholesterol levels, the therapeutic role of this drug is to promote anti-oxidative and anti-inflammatory properties that may also have a role in modulating the immune system and potentially contribute to the treatment of neuroinflammatory disorders.[14] Interestingly, chronic inflammation is often linked to degenerative conditions, and a common outcome of this condition is cognitive dysfunction, as seen in Alzheimer's disease.[15] Clinical trials and meta-analyses are still limited in number, but there is a report that prior exposure to statins can have a protective effect on the development of sepsis and decrease mortality in critically ill surgical patients.[16] However, recent epidemiological studies failed to prove such an effect on mortality rate.[17]

Therefore, the studies are still controversial and data collection cannot yet provide conclusive evidence. There are recent reports about the benefits of statins in experimental sepsis and endotoxemia in peripheral organs. However, little is known about their effects on the central nervous system (Figure 1). In rats surviving sepsis that had received a pretreatment of simvastatin we did not see such behavioral alterations, including memory impairment seen in rats that had not received the simvastatin pretreatment.[18] These results suggest that patients taking statins and going through sepsis should not stop taking this medicament, as it seems to protect the brain against oxidative stress. In addition, patients with neurodegenerative disease, such as Alzheimer and Parkinson, might also benefit from simvastatin use. However, more experimental and clinical studies are necessary to test this hypothesis.

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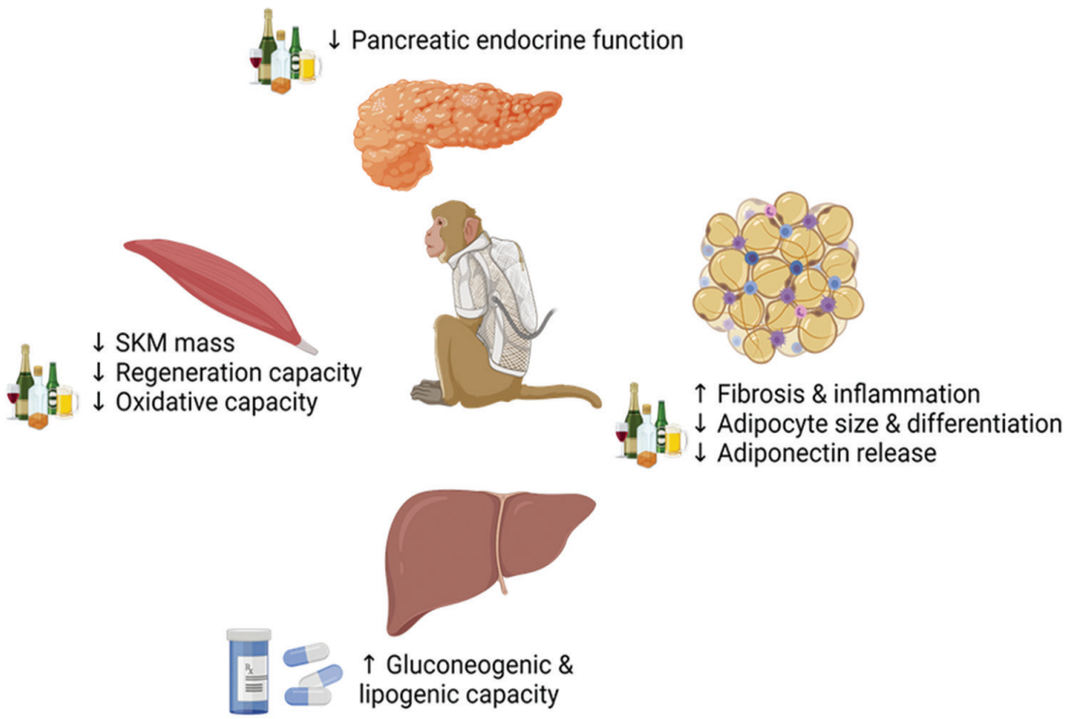

Collectively, data obtained by a team of investigators at the LSU Comprehensive Alcohol Research Center using simian immunodeficiency virus infected non-human primates administered chronic binge alcohol and treated with antiretroviral therapy show marked pathophysiological alterations affecting the endocrine pancreas, skeletal muscle, adipose tissue, and the liver. Pro-inflammatory/oxidative and pro-fibrotic environment, together with significant bioenergetic dysfunction in immune- and non-immune cells contribute to the increased risk for comorbidities that form part of the geriatric syndromes. Our findings indicate multiorgan contribution to the metabolic dyshomeostasis seen in alcohol-treated SIV-infected macaques, which our more recent studies show is also present in persons living with HIV. Alcohol bottles next to specific processes indicates that the changes are predominantly associated with alcohol. Medication vial and pills next to specific processes indicates that the changes are predominantly associated with antiretroviral therapy in infected macaques, treated with alcohol. Courtesy: Patricia E. Molina, Louisiana State University Health Sciences Centre, New Orleans, USA.

Environmental and Behavioral Modifiers of Comorbidities in Persons Living with Human Immunodeficiency Virus

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Introduction

Alcohol use disorder (AUD), the most common and costly form of substance use disorder in the United States,[1,2] contributes to approximately 3.4% of global non-communicable disease-related burden of deaths, 5% of net years of life lost and 2.4% of net disability-adjusted life years, with higher burden for cancer and liver cirrhosis.[3] Several lines of evidence support a dose–response relationship between at-risk or unhealthy alcohol use and incidence of several comorbidities including diabetes mellitus, cardiovascular disease, and infections.[4]

Human immunodeficiency virus (HIV) mortality and new infections have decreased during the past decade, and people living with HIV (PLWH) receiving antiretroviral therapy (ART) have reduced HIV-associated morbidity and mortality, making HIV a chronic disease. Unhealthy alcohol use and HIV frequently coexist.[5–7] Research in the Louisiana State University Health Sciences Center Comprehensive Alcohol-HIV/AIDS Research Center focuses on the interaction of alcohol with underlying pathophysiological mechanisms of HIV infection disease progression.

Pathophysiological Effects of Alcohol in the Context of Immunodeficiency Virus Infection

Unhealthy alcohol use produces significant multisystemic pathophysiological alterations, including disruption of nutritional, metabolic, oxidative, and neuroendocrine pathways.[8] Most of our knowledge is derived from studies conducted in simian immunodeficiency virus (SIV)-infected rhesus macaques, the best animal model for studying the pathogenesis of HIV-like infection. We have examined the impact of chronic binge alcohol (CBA) administration on the host response to SIV infection in rhesus macaques.[9] Our results[10–12] show a significant temporal acceleration to end-stage disease in the absence of ART, with consistently higher plasma, cerebrospinal fluid, and tissue viral loads among CBA-treated animals compared to controls.[13] Our data show that CBA increases virus infectivity following intrarectal inoculation, alters mucosal immunity, and accelerates progression of disease and tissue injury in non-ART treated macaques. ART leads to a similar reduction in viral load in CBA and control animals, suggesting that, with strict adherence, ART may effectively control viremia.[14] However, emerging (unpublished) data suggest that viral expression in reservoirs remains elevated in ART-treated, CBA-administered, SIV-infected macaques, a finding which cannot be explained by decreased adherence and that highlights the possibility that viral reactivation in PLWH may be more common in heavy drinkers than in non-drinkers.

Mechanisms Underlying Alcohol-mediated Pathogenesis

Significant evidence points to gut immunopathology as a central factor in alcohol-mediated pathogenesis in SIV infection. Our findings show that CBA increases intestinal

lymphocyte turnover, decreases the total number of lymphocytes in the small intestine, and increases the percent of gut mucosal HIV target cells.[13,15-17] The decrease in gut lymphocyte numbers is associated with marked increases in both CD4+ and CD8+ T cell proliferation coupled with increased gut mucosal CD8+ T cell death. These intestinal mucosa events adversely affect mucosal barrier function, allowing leak of bacterial toxins into the systemic circulation. Results from our recent studies show higher circulating levels of soluble CD14, a marker for gut leak in CBA-treated, SIV-infected macaques, as compared to sucrose controls. This enhanced gut leak leads to immune activation as shown by the significantly higher percentage of activated and senescent CD8+ T cells in non-ART CBA/SIV macaques, and this appears to be restored by ART treatment.[18] More recent studies in PLWH participating in the New Orleans Alcohol Use in HIV (NOAH) longitudinal study show that sCD14 levels positively correlate with AUDIT scores in PLWH and that PLWH with higher AUDIT scores have a significantly greater percent of activated immunosenescent CD8 T cells as compared to subjects with low AUDIT scores, despite being virally suppressed. Integrating those findings, we have developed a working model in which binge alcohol consumption in the infected host leads to increased viral replication, lymphocyte turnover, dysbiosis, and gut barrier leak resulting in translocation of toxins and bacterial products into the systemic circulation Figure 1. This promotes chronic immune activation, leading to a state of immune exhaustion and senescence and subclinical inflammation that contributes to tissue injury and the development of comorbidities in PLWH including cancer,[19,20] chronic liver disease,[21] reactivation of opportunistic viruses,[22] renal failure,[23] cardiovascular disease,[24] diabetes,[25] lipodystrophy,[26] and osteoporosis;[27] all of which are associated with accelerated biological aging and frailty.

Clinical data show that lifetime alcohol exposure positively associates with the phenotypic frailty index after adjustment for subject demographics, HIV-related covariates, smoking, and history of other substance misuse.[28] At the core of the frailty-related phenotype (unintentional weight loss, exhaustion, weakness, slow walking speed, and low physical activity) is loss of muscle mass and metabolic dysregulation, which has been the focus of research in the Molina lab. Our data demonstrated accentuated end-stage wasting in CBA/SIV macaques that was associated with amplified localized skeletal muscle (SKM) inflammation, profound depletion of antioxidant capacity (oxidative stress), increased proteasomal activity,[29] and decreased myoblast differentiation potential.[30,31] In addition to enhanced SKM protein degradation and impaired myogenic potential, we observed impaired anabolic signaling and decreased whole-body insulin sensitivity, despite viral suppression with ART.[32]

Our data show that CBA dysregulates global regulatory gene networks associated with SKM wasting and this was associated with marked alterations in SKM extracellular matrix.[33,34] CBA impairs SKM regeneration potential in asymptomatic SIV-infected ART-treated NHPs as reflected by decreased myotube formation and myogenic gene expression in primary myoblasts and this is associated with decreased SKM and circulating micro RNA (miR)-206 expression. Mechanistic studies strongly suggest decreased miR-206 and the resulting increase in histone deacetylase 4 (HDAC4) expression as central factors in CBA- and ART-associated impaired myogenesis and differentiation.[35]

Our data also identified marked changes in anabolic signaling in SKM including reduced phosphatase and tensin homolog (PTEN), a phosphatase that attenuates insulin signaling, and total mammalian target of rapamycin and ribosomal protein S6 (rpS6) protein expression. These alterations in anabolic signaling were associated with dysregulation of mitophagy-related and anti-apoptotic gene expression in myoblasts isolated from SIV-infected nonhuman primates that were associated with altered mitochondrial function.[36] These findings suggest that CBA and SIV infection disrupt mitochondrial homeostasis and are supported by our finding that SKM oxidative capacity (reflected by decreased succinate dehydrogenase activity) is significantly attenuated in CBA-treated animals. We hypothesize that impaired mitochondrial homeostasis may contribute to the underlying pathophysiology of alcoholic and HIV-associated myopathy.

More recently, data suggest that CBA and ART produce differential (organ-specific) effects that extend beyond SKM and that together may increase risk for metabolic dyshomeostasis.[37] In the liver of SIV-infected NHPs, ART increased gene expression of key gluconeogenic and fatty acid synthetic enzymes, and tumor necrosis factor alpha. In mesenteric adipose tissue, CBA resulted in smaller adipocyte

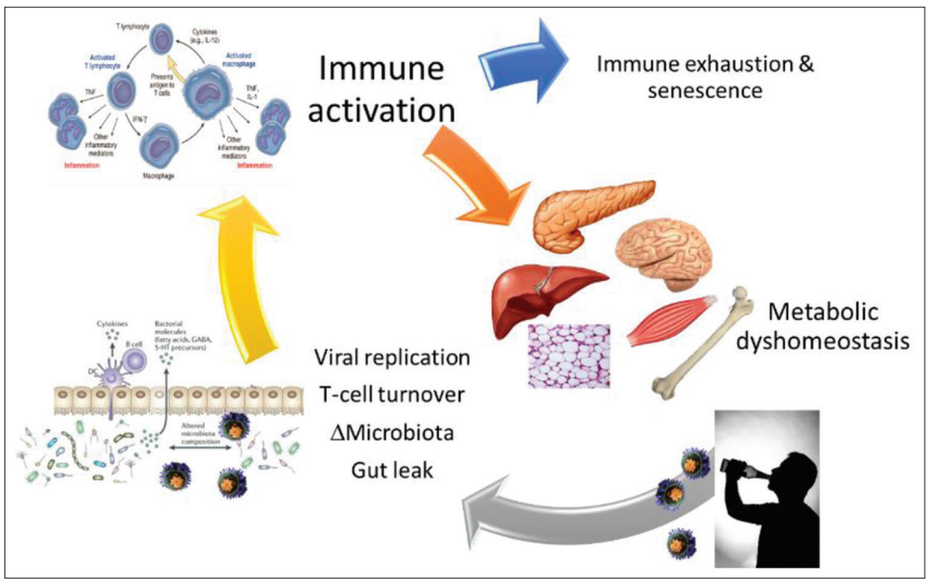

Figure 1: Our pre- and clinical data support the scientific premise that alcohol-enhanced early gut mucosal immunopathology promotes gut leak and chronic systemic immune activation that synergizes with ART to disrupt cellular and tissue metabolic stability.

size compared to that of SUC/SIV macaques, irrespective of ART. Reduced adipocyte size was associated with significantly greater number of mast cells, immune cell infiltration, and collagen staining. We predict these changes may decrease adipose derived-stem cell differentiation, further contributing to metabolic dyshomeostasis.

Our data show that CBA/SIV ART-treated male macaques have significant metabolic dyshomeostasis as measured by frequently sampled intravenous glucose tolerance test with a third-phase insulin infusion (modified minimal model). Data showed lower insulin sensitivity, decreased disposition index (an indicator of insulin resistance), and of these alterations occurred markedly reduced endocrine pancreas response to glucose.[32] All in the absence of fasting hyperglycemia or hyperinsulinemia. Furthermore, we observed decreased adipokine levels, and this could contribute to alcohol-induced metabolic dysregulation.[38-40] Decreased glucose utilization, impaired pancreatic endocrine function, and decreased circulating adiponectin in CBA/SIV macaques, irrespective of ART status indicates multi-organ involvement in the pathophysiology of subclinical metabolic dyshomeostasis.[37]

These changes were seen despite a lack of differences in HOMA-IR (IR) values, body weight, total body fat, abdominal fat, and total lean mass in ART-treated adult NHP fed a nutritionally complete diet. These data[32] highlighted the subtle functional impairment of integral components of the metabolic homeostatic axis (liver–adipose–pancreas–muscle) in CBA/SIV/ART macaques. Metabolic dyshomeostasis under controlled (diet, ART, and unstressed) conditions supports the scientific premise that their prevalence in an aging diet and comorbid conditions is likely higher. That is the focus of our ongoing studies.

Current challenges and future directions

In summary, our collective data support the scientific premise that chronic unhealthy alcohol consumption promotes gut immunopathogenesis resulting in gut leak and immune activation that underlies subclinical chronic inflammation promoting cellular energy metabolism dyshomeostasis in control (brain and pancreas), effector (liver and adipose),

and target (musculoskeletal) organs. Our working hypothesis is that altered cellular energy homeostasis underlies the increased risk for comorbidities in PLWH.

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Status Report of Research on the Skeletal Muscle Metabolism

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In the previous IUPS report on the status of physiology Dr. Janet Taylor reported extensively on the publication trends in the physiology of musculoskeletal system and its control.[1] This report concentrates on a narrower field of the locomotion research, that is, skeletal muscle metabolism during the past 5 years. Skeletal muscle as a large and multifunctional organ that, –for example, in addition to contractile function – produces and secretes substances with hormone-like regulatory functions.[2] Skeletal muscle is involved in various processes related to health and disease.[3] Furthermore, there is increasing amount of information how skeletal muscle adapts to different stimuli, such as exercise and nutrition.[4,5] Large scale methodologies such as transcriptomics, proteomics, and metabolomics are producing huge amounts of information that help us to understand complex relations between cells, tissues, and organs, the physiology of the whole organism.

A search with the term "skeletal muscle" confined to research articles in the Web of Science (WoS) Core Collection produced 156,450 publications in the years 1945–2019. Additional term "metabolism" limited the number of publications to 26,413. Respective numbers in the past 5 years (2015–2019) were for "skeletal muscle" 33,712 and for the additional term "metabolism" 6894. There was a slight yearly increase in these publications ranging from 1611 in 2015 to 1840 in 2019. Interestingly, over 60% out of these 8,544 articles were Open Access publications showing that OA publishing is gaining popularity.

A search including the years 2015–2019 with the term "health" produced 698,179 research articles and 13,657 articles using additional term "metabolism" and 652 articles with a further term "skeletal muscle." Similar search with the initial term "disease" produced 963,978 research articles. 41,753 articles were found with an additional term "metabolism" and 1657 papers with a further term "skeletal muscle." These figures show that skeletal muscle metabolism is studied considerably more in disease – than health-related research. It must be noted that these figures are suggestive since the used initial search terms produce somewhat overlapping results.

In 2015–2019 articles found with the term "skeletal muscle" fall mainly to WoS Categories Physiology, Biochemistry Molecular Biology, Cell Biology, Endocrinology Metabolism, Sport Sciences, and Neurosciences. When adding the term "metabolism," most publications fall to WoS Categories Physiology, Endocrinology Metabolism, Biochemistry Molecular Biology, Sport Sciences, Nutrition Dietetics, and Cell Biology. When searching with the terms "health," "skeletal muscle," and "metabolism," the main WoS Categories were Endocrinology Metabolism, Nutrition Dietetics, Physiology, Biochemistry Molecular Biology, and Cell Biology. When searching with terms "disease," "skeletal muscle," and "metabolism," the main WoS Categories were Biochemistry Molecular Biology, Endocrinology Metabolism, Cell biology, Physiology, and Nutrition Dietetics. It can be noted that the proportion of diet and nutrition research is more emphasized in the disease- and health-related research than in skeletal muscle research in general. Another notion is that the Category Sport Sciences seem not to have an important role in health- and disease-related skeletal muscle research. However, when inspecting the publications in both areas, there is a good number of articles where the effects of exercise or physical activity in general were studied.

According to WoS, during the past 5 years published highly cited research articles on skeletal muscle metabolism in normal and disease conditions [Figure 1] reveal that there is high interest on mitochondrial metabolism,[6-8] amino acid, and fatty acid metabolism (e.g.,[9,10] effects of diet,[11,12] organ cross-talk,[13] irisin,[14] and inflammation).[15]

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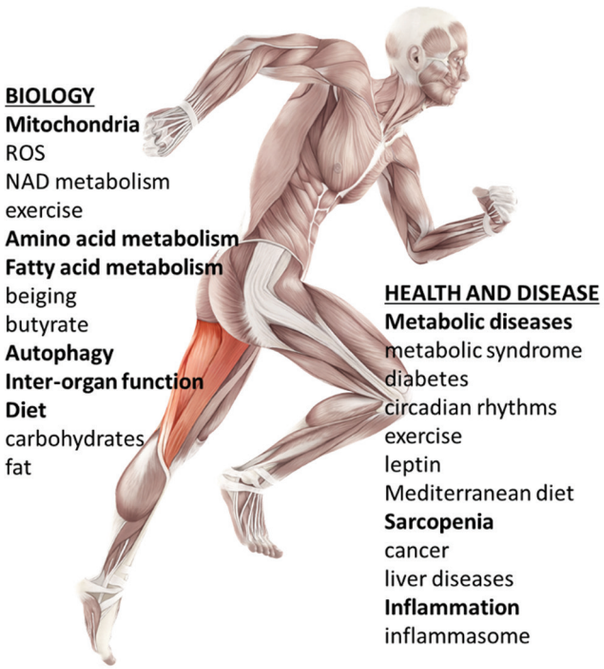

Figure 1: Research topics of interest in 2015-2019 in skeletal muscle metabolism based on the highly cited publications by Web of Science.

Recent review articles provide comprehensive views on the current research and also suggest future trends for research in skeletal muscle metabolism. The topics of interest in research articles are naturally often themes of these reviews. Some of the interesting themes also in the near future are lipid metabolism, especially the role of lipid droplets,[16] metabolic flexibility in health and disease,[5] epigenetics,[17] the role of ROS metabolism,[18] carbohydrate and amino acid metabolism,[19,20] and sarcopenia.[21] Still developing omics techniques (metabolomics, fluxomics, proteomics, transcriptomics, and lipidomics) will provide exciting insights to the mechanisms of metabolism in health and disease.[5,22-26]

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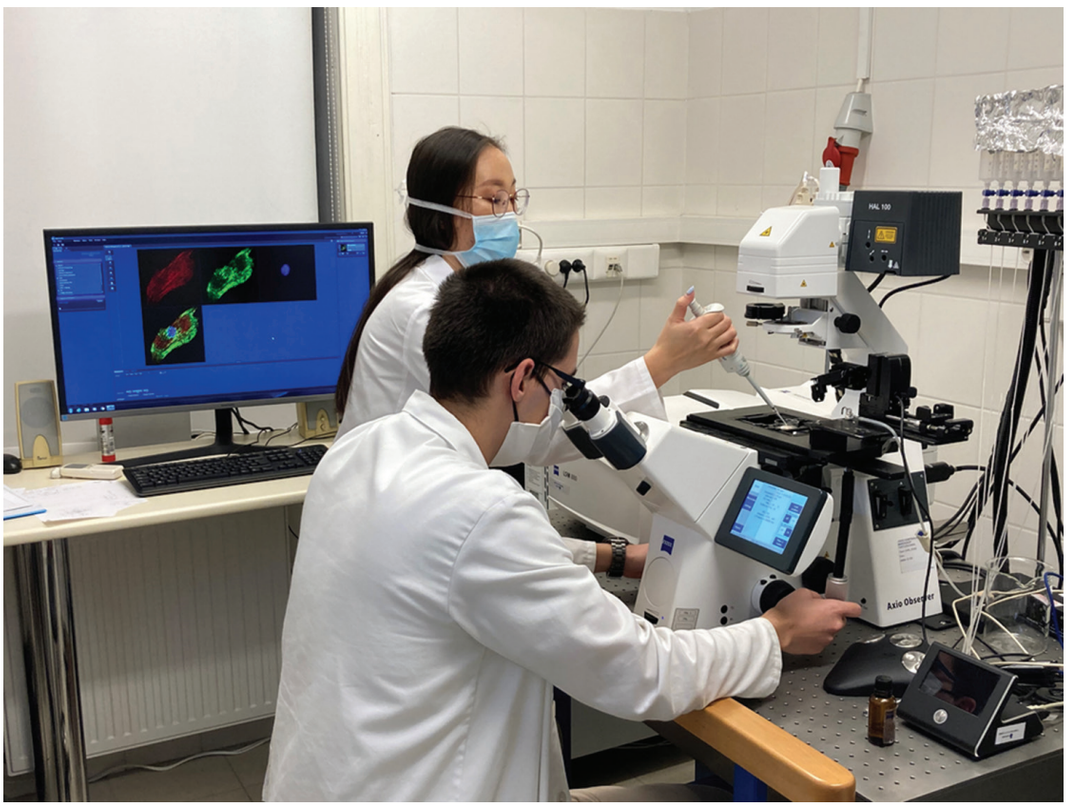

Students of the Doctoral School of Molecular Medicine at the Department of Physiology, University of Debrecen, Hungary studying the cytoskeleton of the myogenic C2C12 cells using confocal microscopy at a high spatial resolution. Cell were stained for actin (red; Cy3) and Septin7 (green; Alexa Fluor 488) with fluorescently labeled antibodies. The cell nuclei are also visualized (blue; DAPI). Courtesy: Laszlo Csernoch. University of Debrecen, Debrecen, Hungary.

Dietary Supplements that Positively Influence Skeletal Muscle Function by Regulating the ROS-rich Environment

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Age-related decline of skeletal muscle function (sarcopenia) is due to the loss of muscle mass and strength.[1] This disadvantageous alteration can be the consequence of several factors, including oxidative stress that is the result of the accumulation of reactive oxygen and nitrogen species (ROS/RNS). At physiological concentrations ROS/RNS play essential roles in a variety of signaling pathways. There is an optimal level of ROS/RNS to sustain both cellular homeostasis and adaptive responses, and both too low and too high levels of ROS/RNS are detrimental to cell functions.[2]

Skeletal muscle consumes large quantities of oxygen and can thus generate large amounts of ROS and as well as reactive nitrogen species. Mitochondria are one of the most important sources of ROS in the skeletal muscle. The origin of the increased ROS production and oxidative damage with aging is mitochondrial dysfunction,[3] caused by age-related mitochondrial DNA mutations, deletions, and damage,[4] as well as the impaired ability of muscle cells to remove dysfunctional mitochondria.[5]

Proteins, just as other biomolecules, are frequently affected by oxidation. Elevated ROS levels can cause reversible or irreversible posttranslational modification of cysteine, selenocysteine, histidine, and methionine all of which alter protein function. These and other oxidative posttranslational modifications of proteins, for example, carbonylation, are characteristic in the aged muscle.[6]

The gating of the sarcoplasmic reticulum (SR) calcium channel RyR1 is controlled by the transmembrane redox potential of SR. The majority of redox buffers within the cytosol of a muscle cell are based on the relative concentration of oxidized (GSSG) and reduced (GSH) glutathione or NADH and NAD^+.[7] It was shown that glutathione transport across SR/ER membranes is very fast and correlates with the expression of RyR1 in terminal cisternae of the SR.[8] This implies that the closed but not the open conformation of RyR1 senses the changes in the redox state of the myoplasm.

On the other hand, the function of satellite cells, the dormant stem cells responsible for muscle regeneration following injury, is also altered by oxidative stress and, thus, with aging. In the aged muscle, satellite cells exhibit a reduced capacity to proliferate and self-renew. Furthermore, the ROS production is higher in isolated satellite cells from aged than from young muscle. In addition, a decline in the antioxidant capacity of satellite cells was also observed with age, thus diminishing satellite cell function with increased ROS levels.[9]

As the trace element selenium has an antioxidant property it plays an important role in several muscle functions, and associated enzymes as glutathione reductase and peroxidase protect muscle fibers from reactive oxygen species.[10] Not surprisingly, skeletal muscle disorders manifesting in muscle pain, fatigue, proximal weakness, and serum creatine kinase elevation have been reported in patients with selenium deficiency.[11]

The biological functions of selenium are associated with selenoproteins [Figure 1], a family of proteins which contain this micronutrient in the form of selenocysteine. Most of those selenoproteins whose function has been identified are catalytically active in redox processes. This includes glutathione peroxidase (GPx) which plays a pivotal role in the catalytic decomposition of H_2O_2 into H_2O and oxygen. In mammals, there are a number of selenocysteine-containing GPx-s and they modify the oxidation of reduced glutathione.[12]

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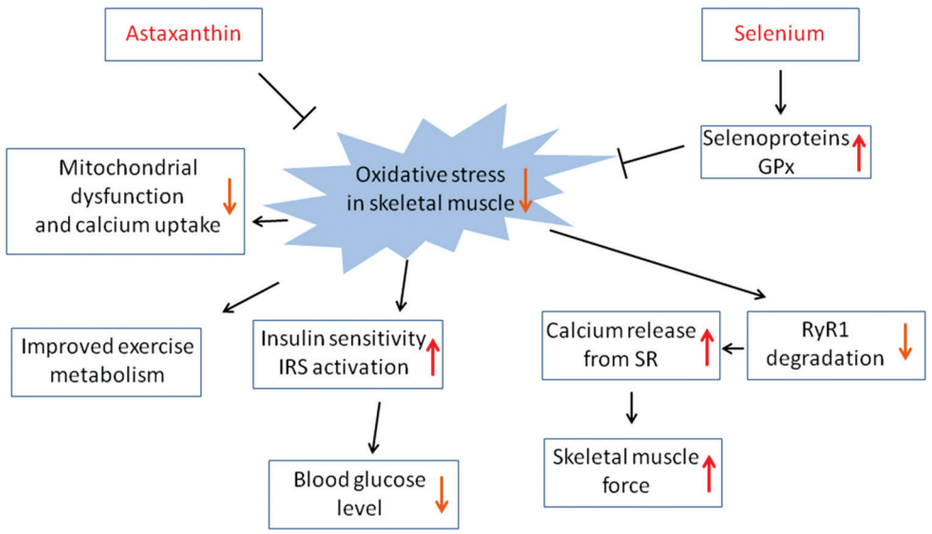

Figure 1: Schematic summary of comprehensive and favorable effects of astaxanthin and selenium compensating for the oxidative stress in skeletal muscle.

In our previous work on young mice, we have shown that selenium supplementation increases calcium release from the SR and thus improves *in vivo* and *in vitro* skeletal muscle performance. These effects were accompanied by the elevation of Selenoprotein N levels, encoded by the SEPN1 gene, which could result in enhanced oxidative stress tolerance during long lasting contractions.[13] Moreover, selenium supplementation was able to compensate for the reduction in muscle force and SR calcium release that accompanies aging. While the Selenoprotein N content of the muscle is known to decrease with age[14] our experiments have demonstrated that selenium supplementation is also able to significantly reverse the decline of Selenoprotein N content in aged muscle.[15]

Carotenoids have gained special interest during the last decades, due to their strong antioxidant, repairing, anti-proliferative, anti-inflammatory, and potential anti-aging effects. They can be used to prevent oxidative stress-related diseases and chronic inflammation [Figure 1]. Astaxanthin as a xanthophyll is one of the most powerful carotenoid. Astaxanthin is a fat-soluble nutrient (it incorporates into cell membranes) with increased absorption when consumed with omega-3-rich seed oil; however, it cannot be converted to Vitamin A and therefore cannot support retinol-specific processes such as vision. With its unique molecular structure astaxanthin stretches through the bilayer membrane, providing resilient protection against oxidative stress. It can scavenge and quench ROS and free radicals (superoxide anion, hydrogen peroxide, singlet oxygen, etc.).[16]

The oxidative stress can also lead to insulin resistance by activating various kinases including JNK, which catalyzes the phosphorylation of serine residues in IRS-1 inhibiting its activity and preventing its interaction with the insulin receptor. Furthermore, oxidative stress switches the GLUT4 sorting to the degradation of GLUT4 vesicles. Dietary astaxanthin administration was effective in patients with type-2 diabetes mellitus, because it improves insulin sensitivity, IRS-1 activation, Akt phosphorylation, and GLUT4 translocation in skeletal muscle leading to increased insulin sensitivity and a decrease in blood glucose level.[17]

In mouse exercise experiments, supplementation with astaxanthin can effectively improve the side effects of exercise metabolism and the individual's performance and recovery. Four weeks of astaxanthin treatment in mice prolonged

the running to exhaustion. During exercise, astaxanthin administration facilitated lipid metabolism instead of glucose utilization, which improved the endurance and reduced adipose tissue.[18] Oxidative stress-induced modification of lipid peroxidase carnitine palmitoyltransferase I (CPT-I) was reduced with the application of astaxanthin.[19] Liu *et al.*[20] also suggested that astaxanthin intake increases the PGC-1α level in skeletal muscle leading to the acceleration of lipid utilization by the activation of mitochondrial aerobic metabolism during exercise. In oxidative-type soleus muscle, 45 days of astaxanthin supplementation resulted in a mitochondrial-targeted action, as the treatment increased glutathione content in the mitochondria during exercise, limited oxidative stress, and delayed exhaustion in Wistar rats.[21]

In our experiments, the body weight gain was smaller, while the maximal grip force was increased following 4 weeks of astaxanthin diet. Astaxanthin supplementation also increased *in vitro* tetanic force, without affecting excitation-contraction coupling and exerted a protecting effect on the mitochondria. Our results seem to agree with the hypothesis that astaxanthin supplementation favorably alters mitochondrial, and attenuates activity-dependent mitochondrial calcium unbalance by possibly scavenging ROS and also by down-regulating mitochondrial calcium uptake.[22]

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Hypothalamic Regulation of Oxytocin Neuron Activity in Pregnancy and Lactation

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Oxytocin is best characterized for the regulation of birth and lactation.[1] Under basal conditions, circulating oxytocin levels are relatively constant[2] but rise progressively over the course of pregnancy, with large pulses evident during birth.[3,4] These pulses trigger rhythmic contraction of the uterus, but only when myometrial oxytocin receptors are upregulated at the end of pregnancy.[5] The Ferguson Reflex is established during parturition whereby uterine contractions induce further oxytocin secretion through positive feedback, which is terminated once birth is complete.[6,7] Although oxytocin is not essential for birth, it is required for the normal timing of birth.

Oxytocin is synthesized by magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei as well as in several smaller accessory nuclei.[1] Oxytocin neurons each project a single axon to the posterior pituitary gland where they arborize extensively to several thousand terminals filled with neurosecretory vesicles containing oxytocin, which is released into the circulation by exocytosis.[8] Oxytocin secretion from the posterior pituitary gland is triggered by action potential (spike) invasion of the axon terminals. Under basal conditions, oxytocin neurons typically fire action potentials at 1–5 spikes s^{-1} to maintain circulating oxytocin levels at a stable level.[1] However, immediately before each uterine contraction during birth, all oxytocin neurons simultaneously fire high frequency bursts at up to 100 spikes s^{-1} every 5–10 min.[9] The bursts last only 1–2 s and are superimposed on the low frequency firing, but the intra-burst firing rate is so high that each burst releases a large pulse of oxytocin into the circulation. After each burst, oxytocin neurons typically fall silent for several seconds, and then resume their low frequency firing until the next burst occurs some minutes later. Burst firing of oxytocin neurons is maintained during lactation to trigger episodic milk ejection during suckling.[10]

The oxytocin system undergoes remarkable plasticity over the course of pregnancy to favor burst firing during birth and lactation; this includes changes in the morphology and properties of oxytocin neurons, changes in surrounding astrocyte morphology and function as well as changes in their afferent inputs.[11-13] We have recently shown that intracerebroventricular kisspeptin consistently increases oxytocin neuron firing rate in late-pregnant rats but has no effect on oxytocin neuron firing rate in virgin, early-pregnant, or mid-pregnant rats.[14,15] We now know that this kisspeptin excitation occurs through a local action in the supraoptic nucleus (Abbasi, Perkinson, Iremonger and Brown, unpublished) but not by a direct action of kisspeptin on oxytocin neurons (Seymour, Piet, Campbell and Brown, unpublished).

Kisspeptin neurons are located in the hypothalamic arcuate nucleus, anteroventral periventricular nucleus, and periventricular nucleus in rodents[16] and project widely throughout the brain,[17] including the supraoptic and paraventricular nuclei.[18] Remarkably, we found a substantial increase in the density of kisspeptin expression in fibers surrounding the supraoptic nucleus in late-pregnant rats that arose exclusively from the periventricular nucleus.[14] While the total number of neurons that project from the periventricular nucleus to the supraoptic nucleus was unchanged in late pregnancy, more of these neurons expressed kisspeptin, suggesting that they increase kisspeptin expression in late pregnancy.

While some kisspeptin fibers coursed into the supraoptic nucleus, the densest labeling was in the perinuclear zone surrounding the supraoptic nucleus, which contains glutamatergic and GABAergic interneurons that project into the supraoptic nucleus. However, kisspeptin does not affect the frequency or amplitude of glutamatergic or GABAergic post-synaptic potentials in supraoptic nucleus neurons in brain slices from virgin or late-pregnant rats (Seymour, Piet, Campbell and Brown, unpublished), suggesting that the perinuclear zone projections to oxytocin neurons are not activated by kisspeptin. Nevertheless, the periventricular nucleus kisspeptin projection might excite oxytocin neurons through presynaptic modulation of other afferent inputs, such as brainstem noradrenergic inputs that are activated during birth.[19] Taken together, our new data show that kisspeptin projections from the periventricular nucleus to the oxytocin system increases over pregnancy and excites oxytocin neurons at the end of pregnancy.

In early pregnancy, the anterior pituitary gland secretes twice-daily pulses of prolactin that maintain pregnancy,[20,21] but by mid-late pregnancy placental lactogens take over this role and inhibit anterior pituitary prolactin secretion by inhibition of hypothalamic prolactin receptors.[22] Oxytocin neurons express prolactin receptor mRNA,[23] and prolactin administration increases phosphorylation of signal transducer and activator of transcription 5 (STAT5)[24] in oxytocin neurons of virgin female rats.[25] The most highly upregulated genes in the supraoptic and paraventricular nuclei of late-pregnant rats are those that encode for suppressors of cytokine signaling,[26] which are intracellular inhibitors of the STAT5 pathway,[27] suggesting that prolactin signaling to oxytocin neurons is increased during pregnancy. Indeed, on the expected day of birth, phosphorylated STAT5 (pSTAT5) is expressed by almost all oxytocin neurons but is almost absent from oxytocin neurons in virgin rats.[26] Chronic prolactin receptor activation increases the expression of oxytocin mRNA[28] and increases the number of oxytocin-positive neurons detectable by immunohistochemistry.[29] Hence, it appears that high placental lactogen in mid-late pregnancy might promote oxytocin synthesis required to cope with the increased secretory demands of birth and lactation.

pSTAT5 is also present in almost all oxytocin neurons in lactating rats.[26] which prevents further activation by exogenous prolactin.[29] Pup removal and hence the removal of the suckling stimulus, also lowers oxytocin mRNA expression in lactating rats and this reduction is prevented by prolactin administration.[30] It, therefore, appears that prolactin continues to stimulate oxytocin mRNA expression during lactation to mediate the suckling-induced synthesis of oxytocin.

Prolactin rapidly and transiently inhibits the activity of oxytocin neurons in virgin rats[23] and this inhibition is likely through activation of potassium channels.[31] Remarkably, the effect of prolactin on oxytocin neuron activity depends on reproductive status; we found that prolactin administration to lactating rats increases the firing rate of ~20% of oxytocin neurons[29] when these hormones must be secreted together for milk synthesis (prolactin) and milk ejection (oxytocin).[1,32] Removal of the suckling stimulus also decreases circulating oxytocin concentrations to virtually zero and this change is also prevented by prolactin administration,[30] suggesting that prolactin mediates suckling-induced oxytocin release during lactation. While not all oxytocin neurons are excited by prolactin in lactation, its effects are sufficient to increase oxytocin release in lactating rats, perhaps also by prolactin action at the axon terminals in the posterior pituitary gland.[33]

The attenuation of prolactin-induced oxytocin neuron inhibition evident in lactating rats could be explained by reduced activation of the potassium leak conductance but this cannot account for the prolactin-induced excitation seen in some neurons.[29] The expression of prolactin receptors is similar in oxytocin neurons of virgin, pregnant, and lactating rats[34] and prolactin-induced pSTAT5 is similar in oxytocin neurons from virgin and lactating rats.[29] Hence, in lactation, the switch from prolactin inhibition to excitation of oxytocin neurons is unlikely to be mediated by a change in prolactin receptor expression or by desensitization of STAT5 signaling. The mechanism by which prolactin excites some oxytocin neurons during lactation is still unknown but might involve activation of depolarizing TRPV channels on oxytocin neurons, as has been shown for prolactin in sensory neurons of female rats.[35]

Concluding Remarks

The emergence of an excitatory kisspeptin projection to the oxytocin system and the switch from prolactin inhibition to

excitation of oxytocin neurons in lactation add to the suite of regulatory mechanisms that prepare the oxytocin system for normal birth and lactation [Figure 1]. These newly-characterized regulatory mechanisms might provide a platform for the development of new therapeutic targets for managing complications in pregnancy and lactation. Antagonism of the excitatory projection from kisspeptin neurons to the oxytocin system might reduce the excitability of oxytocin neurons near term and thus reduce the risk of preterm labor. If early activation of oxytocin neurons results from early- and/or over-activation by placental lactogens, elevated placental lactogens might have potential for use as a biomarker for pregnancies at risk of pre-term delivery.

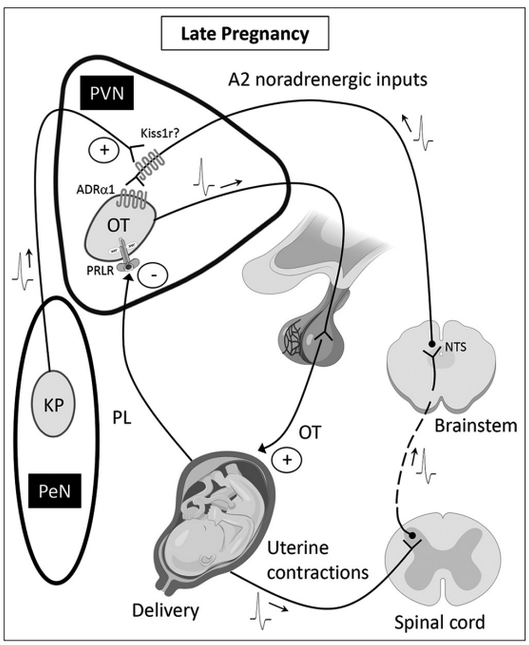

Figure 1: Regulation of oxytocin neuron activity in pregnancy. In late pregnancy, kisspeptin expression increases in periventricular nucleus projections to the paraventricular and supraoptic nucleus PVN/SON (SON not shown on schematic). We hypothesize that kisspeptin increases oxytocin neuron activity at parturition through A2 adrenergic inputs from the brainstem nucleus tractus solitarius, which have been shown to activate oxytocin neurons at this time. An increase in coordinated action potential firing in oxytocin neurons causes the release of oxytocin from the posterior pituitary gland, which is then released into the circulation to activate myometrial oxytocin receptors, causing uterine contractions. In turn, uterine contractions elicit positive feedback stimulation of oxytocin burst firing through brainstem noradrenergic inputs. We hypothesize that placental lactogen secretion excites oxytocin neurons at parturition (as it does in lactation) to facilitate burst firing for successful parturition.

Acknowledgments

The authors thank Ms Chantelle Murrell for preparation of Figure 1.

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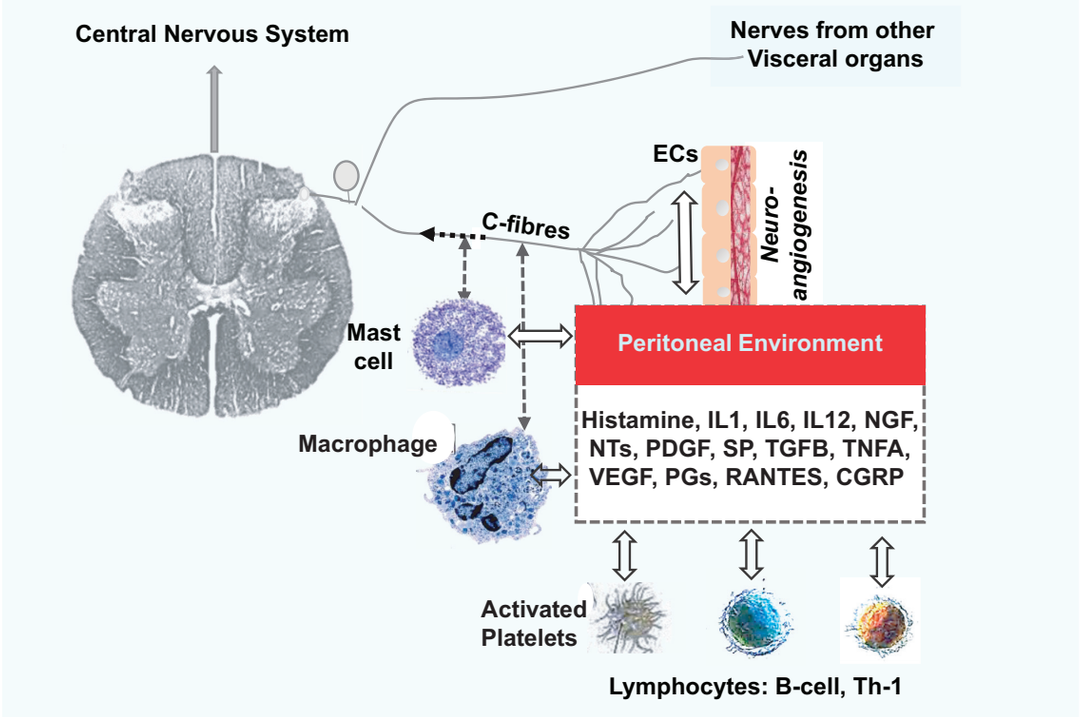
Peripheral mechanisms putatively involved in invoking endometriosis-associated pain



Adapted from: Ghosh et al. (2020) Reprod. Med., 1, 32–61. ECs, ectopic cells. Image credits: Wikimedia Commons. Courtesy: Debabrata Ghosh, All India Institute of Medical Sciences, New Delhi, India.

Integrative Approach to Understand Endometriosis and Associated Pain, Infertility, and Cancer

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"*...complexity is something that most biologists try to avoid.*"[1]

Most probably, Daniel Shroen's 1690 reference to typical ulcers on the surfaces of the bladder, intestines and broad ligaments were those of endometriosis lesions. Then, Karl Von Rokitansky in 1860 described the disease before Sampson who came up in 1927 with his authoritative and nicely illustrated extensive paper on endometriosis.[2] Remarkably, Brotherson[3] in 1776 wrote regarding endometriosis, "In its worst stages, this disease affects the well-being of the female patients totally and adversely, her whole spirit is broken, and yet she lives in fear of still more symptoms such as further pain..." In the last two and half century, there has been little change; women with endometriosis suffer as much; the cause of endometriosis is still unknown; there is no definitive cure and surgical outcome is variable and it often recurs causing long-term problems for the patient, patient's family, and her physician.

Endometriosis is one of the most common gynecological conditions. About 200 million women in the reproductive age worldwide suffer from endometriosis and it can also occur at a young age. Endometriosis is often associated with objective pain components in form of dysmenorrhea, pain at ovulation, dyspareunia, abnormal bleeding, chronic pelvic pain and fatigue, and infertility – an issue which is often under-diagnosed. In fact, the prevalence of endometriosis can rise even up to 50% in women of reproductive age with infertility and/or pain. It is also associated with moderately higher chance elements of cancers, particularly ovarian cancers. Over all, it has severe impact on women's productivity at work and their long-term physical, mental, and social well-being. The resultant psycho-social and economic burden are substantial.[4,5] Thus, understanding this disease has become a real necessity today.

While the number of publications on "endometriosis" in the PubMed portal has risen from 2500 between 1980 and 1990, to 4500 in 1990's, and then to 6500 in 2000's, and now to more than 10,000 in the last decade, efforts to go beyond the premises of perceived idea of prevalent health-care system that normalizes pelvic pain for women and *a-priori* believes that endometriosis is a benign disorder is a relatively recent happening.[5] It is only recently being perceived that endometriosis is a polygenic, multi-modal, and multifactorial pathophysiological phenomenon.

At the turn of the present millennium, it was predicted that endometriosis susceptibility genes would be identified shortly.[6,7] This resulted in palpable excitement in the research in the genetic basis of endometriosis as reflected in the number of publications. A PubMed search on "endometriosis and polymorphism" hits only 21 papers till 2000, while 80 papers in the next 5 years (averaging 17 papers per year) followed by another 140 papers till 2010 (averaging 28 papers per year) and additional 210 papers till 2015 (averaging 42 papers per year) which, however, was followed by a drop with an average of 29 papers per year till 2019. A strong reason for this overt fall lies in the observation that the effect sizes of genetic markers linked to endometriosis risk is rather small with virtually no predictive power for individual risk.[8] Alleles at the putative "disease genes" often show low detectance and low penetrance, and are not necessarily sufficient to cause disease.[9] Rather, it is now being increasingly perceived that endometriosis is a complex disease, depending on several predisposing, initiating, and propagating factors that trigger the

induction followed by progression process of the disorder.[10-12] An integrative schema of pathogenesis of endometriosis can well be visualized today [Figure 1].

While genes can explain only a fraction of variation in the disease, emergent functions from networks of molecular processes under the regulatory influences of entwined actions of gene and environment form the foundation of the disease edifice.[11-13] Several groups including our group have examined the differential display of genomic and protein expressions in eutopic and ectopic tissues based on large scale exploration revealing some common molecular processes potentially involved in endometriosis, despite the fact that most of the studies failed to sort and classify the samples in the recommended manner.[11-16] In Figure 2, we have made an attempt to comprehensively present the vulnerable processes putatively involved in endometriosis and endometriosis-associated pain and infertility. There are several lines of evidence now to suggest that the process of induction and progression of the disease can be modulated by epigenetic influences on the process dynamics of molecular and biological pathways.[11,12] Further, integrative studies using appropriate animal models and models of comparative and phylogenetic molecular evolution may provide newer questions and leads related to the disease development, its progression and treatment strategies.

Women with unmanaged and untreated endometriosis are at a moderately increased risk for the development of carcinomas of the ovary and uterus with time. Figure 3 presents a model to explain how endometriosis may be driven to malignant potential. *Driver modules* are the key components of biological pathways that are highly relevant to the pathophysiology of endometriosis as these regulate the underlying pathophysiological processes including cellular proliferation, cell survival, epithelial–mesenchymal transition, and angiogenesis, which may lead to fibrosis. Further studies are, therefore, required to identify *the drivers that modulate key events* conducive to the pathophysiology of ovarian cancers from ectopic lesions through atypical endometriosis and the *passenger modules* that may change as a result of the disease pathogenesis.[13] It will

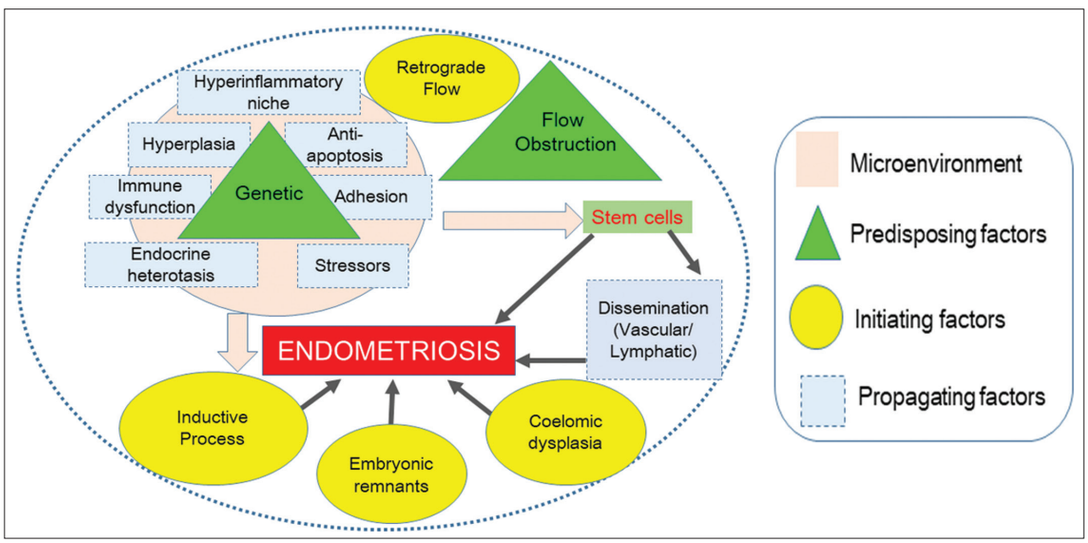

Figure 1: A schema to explain how different factors interact and interlace in the pelvic viscera and cavity (shown as *dotted blue closure*) toward induction and progression of endometriosis. These factors may be classified as predisposing, initiating, and propagating factors depending on how their underlying physiological processes impart on the process of histogenesis resulting in endometriosis. It now appears that emergent processes involving a cohort of molecular processes and their epigenetic modifications underlie the developmental process of endometriosis. Explanatory legends to the key symbols used in the diagram are shown in the box. For further details, Sourial *et al.*,[10] Sengupta *et al.*,[11] and Ghosh *et al.*[13]

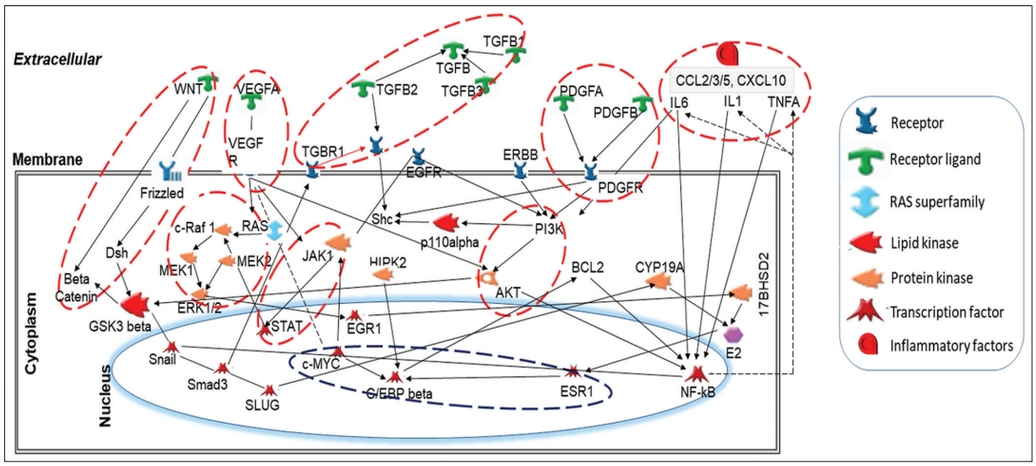

Figure 2: A display of the derived process networks that are putatively dysregulated in eutopic endometrium and ectopic sites in endometriosis. The nodes (shown as *dashed red circles/ellipses*) are known to provide molecular functions of physiological processes such as cell proliferation, epithelial-mesenchymal transformation, angiogenesis, apoptosis, cell survival, steroid hormone responsiveness, inflammatory, and tolerogenic responses and homeostatic stress responses that are known to be affected in the diseased tissue and result in pain and infertility during ovarian endometriosis. Explanatory legends to the key symbols used in the diagram are shown in the box. See Sengupta *et al.*[11] and Ghosh *et al.*[13] for details.

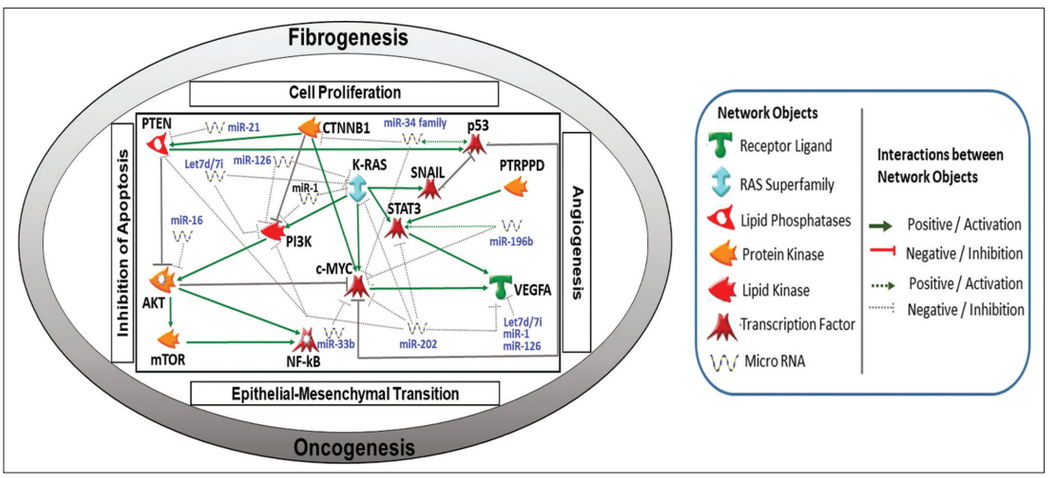

Figure 3: A model of functional network with interactions among driver gene products and microRNAs regulating critical cellular processes leading to fibrotic reaction at ectopic lesions. Cyclic bleeding associated with inflammatory and oxidative stress followed by repeated tissue injury and repair along with recurrent estrogenic stimulation and ovulatory events in the pelvic environment bring about the complex phenotype of neoplastic atypical endometriosis with a trade-off malignant transformation in high risk women population. Integrative studies using different types of appropriate models ranging from animal studies to molecular evolution may provide new leads related to the disease development and its probable progression to ovarian cancer. Explanatory legends to the key symbols used are shown in the box. For further details, see Ghosh *et al.*[13]

indeed be of interest to understand the basis of the greater risk of gynecological cancers among infertile women affected by endometriosis. The impact of heterogeneities at level of tissue, disease and histotypes, and other context-specific determinants that may play important role in the disease development are important and it is necessary to consider them with due weightage in future studies.[13]

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Sex, Gender, Physiological Research, and COVID-19

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As a reproductive physiologist, I study the physiology and pathophysiology of the myometrium. In this short article, I want to touch on how our increasing understanding of reproductive physiology has taken us into areas, I at least, never imagined, and then the fascinating ways our biological sex can influence our physiology, way beyond the obvious. The first point considers the application of our progress in understanding reproductive physiology to post-menopausal women and transgender men becoming pregnant. The second concerns the impact of biological sex on the outcomes of COVID-19 in women and men.

A separation of identity from biology was never envisioned nor was it a motivation when the physiologists Robert Edwards worked with Patrick Steptoe, to bring about *in vitro* fertilization. The world's first "test tube" baby, Louise Brown, was born and headlined throughout the world in 1978. Edwards was awarded the Noble prize for Physiology (or Medicine) in 2010. We now consider IVF and its many auxiliary techniques, such as cryopreservation of eggs, or ovarian tissue, sperm preservation, ICSI, intrauterine embryo transplantation, and embryonic testing, as unremarkable at a population level. They are of course remarkable for the individuals concerned, and their course certainly does not always run smoothly. Physiologists are part of the teams working to improve outcomes from fertility treatments, which remain frustratingly low. It is also the case that the treatments mentioned above are not routinely available or affordable throughout the world.

Our understanding of the basics of reproductive physiology and endocrinology has been tested, and used, to enable women well beyond the menopause to become pregnant and give birth. Headlines around the world periodically hail "the world's oldest mum." The record may be held by Erramatti Mangayamma from India, who was 74 when she gave birth for the first time. She had twins after IVF, three decades after her menopause. These older and often first time mothers demonstrate the enduring longing of many humans to have a child, but also the capacity of the uterus to respond to hormonal environments and an implanting embryo, throughout a woman's life, not just for a few decades. Delivery is usually by cesarean section and so I do not know how contractile the myometrium would be. Based, however, on studies from our group, spontaneous contractile activity can be recorded in non-pregnant women into their 7th decade, suggesting that it might be strong enough.[1] Male reproductive function can also be successfully demonstrated in later life. Old men, some in their 90s, father children, without any medical intervention. That this is usually with wives or partners decades younger, and with less fanfare, moral indignation and ethical debate, is not a physiological discussion.

Our knowledge of physiology has been used to help female-to-male, transgender men conceive, carry a fetus, and become parents. For clarity, I mean here, male gender identity differing from the female sex which was assigned at birth. Most female-to-male transitions occur without the loss of ovaries or uterus. Testosterone is used to stop menstruation and bring about the male secondary sexual characteristics (virilization), such as deep voice, increased muscularity, and facial hair. The desire for a family and genetically related offspring is not restricted to heterosexual couples[2] along with adoption and surrogacy, gestating a fetus with or without help from the fertility methods mentioned above, can enable transgender couples to have a family. The number of transgender men conceiving is not documented but is likely in the thousands The first man to give birth is probably Thomas Beatie in the USA in 2008.[3] Transgender men wanting to conceive will stop taking testosterone, and within around 6 months, menses will recommence.[4] If menstruation recommences on cessation of testosterone, then ovarian function is restored. As with other

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couples, pregnancy may occur at a varying interval after this. The transgender man who had ovaries removed may have stored oocytes or some ovarian tissue ahead of transitioning and taking testosterone.[5] Otherwise, both egg and sperm donors are required, with partners a likely source of one or other, and IVF undertaken. A recent study of transgender and pregnancy found that the majority used their own oocytes and their partner's sperm. There are limited data on whether their previous testosterone use may impair fertility or indeed affect fetal development, but in women, high testosterone may reduce fetal weight.[6] After conception, the pregnancy and delivery outcomes in transgender men progresses as much as that of cis-women (i.e., those whose gender identity and biological sex are congruent). The management of any obstetrical complications will be along best current obstetrical care, and not determined in relation to gender identity or previous use of testosterone.[7] Specific difficulties for such men may not be physiological but rather psychological as their bodies change with testosterone cessation (and pregnancy), and postpartum depression may be more frequent.[8]

As a physiologist working on myometrium, I have not been much troubled thinking about men or male animal models. It is clear, however, that biological sex impacts on so much of our physiology. It is for this reason that more funders and journals are asking for physiological investigations to be performed on female and male animals. This is not just excessive political correctness; there are numerous examples of where the dose and the medicine of choice differ between men and women, due to the effects of biological sex. This can affect adverse reactions and recovery times. It is also the case that the majority of medicines have not been tested on pregnant women (or children). This leads to difficulty in prescribing but also in treating obstetric complications. Specifically, drugs developed to help regulate myometrial contractility, are often unlicensed. The lack of will to test drugs on pregnant women, even for conditions such as preterm labor, means new drugs are not developed. Preterm birth is the leading cause of neonatal death and handicap, and causes huge emotional toll on families and health-care systems worldwide.

The relative protection from cardiovascular disease of being female has long been appreciated. Heart attacks, for example, are uncommon in premenopausal women. Some of this protection is due to estrogen and its vasodilatory effects, and some due to the effect of sex steroid hormones on the renin-angiotensin-aldosterone system (RAAS).[9,10] Both epidemiological and experimental studies have reported sex differences in the therapeutic benefits of modulators of the RAAS pathway, but such differences are often ignored.[11] It is sex differences in parts of RAAS that can account for one of the strangest outcomes of the COVID-19 pandemic – men are more vulnerable than women. More details of some of the below can be found in Wray and Arrowsmith, (2021) Frontiers in Physiology.

Coronaviruses are large enveloped, single-stranded, positive-sense RNA viruses. They contain transmembrane spikes, which have host receptor binding domains and stalks, responsible for membrane fusion and host cell infection. As was found during the 2003 SARS-CoV outbreak, these viruses use the receptor for angiotensin-converting enzyme 2 (ACE2), as their attachment target, as they gain entry to our lungs.[12] That the ACE2 receptor is essential for virus entry was shown using ACE2 knockout mice.[13] For infection, the viral stalks must be activated, and this is achieved by proteases, specifically the host cell's transmembrane serine protease 2 (TMPRSS2),[14] as well as with SARS-CoV-2, an element of self-activation performed by the viral proprotein convertase furin, facilitating its entry into cells.[15] Infection is associated with shedding of ACE2 into plasma and transport thereby to other organs, which if they also express ACE2 receptors, will start to fail. There is downregulation of the ACE2 receptor, which will have physiological consequences.[16] To understand this, we need to remind ourselves of the effects of effects of ACE2 and its relation to the RAAS.[17] Labeled the protective counter arm of RAAS, ACE2 has positive metabolic effects, and is vasodilating, anti-proliferation, and anti-inflammatory, balancing angiotensin II's vasoconstricting role.[18,19] Physiologically, Ang-(1-7) has been shown to signal through a novel GPCR, Mas.[20] As infection produces a downregulation of ACE2, this may contribute to the hypertension and inflammation seen with COVID19, as the vasodilating effect of Ang(1-7) is decreased.[21] Of note here, both ACE2 and TMPRSS2 are modulated by steroid hormones.[22] Although not extensively studied, especially in human tissues, evidence points to sex-based differences in the expression and regulation of ACE2.[23] ACE2 has a wide tissue distribution, beyond lung and is highly expressed in renal, cardiac, fat, and mucosal cells.[24] This tissue-wide distribution probably contributes to the multi organ pathologies brought on by infection. To note, with respect to COVID-19, ACE2 expression is

higher in pneumocytes from men compared to women.[23] In differentiated human airway epithelial cells, treated with vehicle or oestradiol, the latter expressed lower levels of ACE2 mRNA (TMPRSS2 mRNA levels were unaffected). In rats, both sexes have age-related declines in ACE2 expression, but to a greater extent in male rats.[25] Thus, it seems likely that there is sexual dimorphism in the availability of a key infectivity component, ACE2, necessary for COVID-19.

Once epidemiological data on the SARS-CoV-2 pandemic started to be obtained it appeared that there was a sex-based difference in response to the viral diseases, COVID-19; morbidity and mortality were greater in men than women. Subsequent data and analysis have confirmed that being female offers a degree of protection from COVID-19 that persists even when confounders such as, age, infection rates, smoking, social habits, and comorbidities are considered, for example[26] This finding of men succumbing to more severe disease and dying was also a feature in the two previous, smaller coronavirus diseases, Middle East respiratory system (MERS-CoV) in 2012 and SARS-CoV in 2002.[27,28] Statistical information, from 183 countries, the "COVID-19 sex-disaggregated tracker update," from (https://globalhealth5050.org/the-sex-gender-and-covid-19-project/) is recommended; it is updated every 2 weeks.

Our understanding of how female sex steroids affect the distribution and activity of ACE2, and, in turn, how this decreases viral entry into our airway cells helps us understand the decreased risk for females during the pandemic. In addition, if ACE2 receptors are not internalized as ACE2 is shed into plasma, then its beneficial effects, mentioned above, persist in females, for example, blood pressure is better maintained.

Although this is an article focusing on physiology, it would be remiss not to at least overview how sex differences in immunological responses also affect the physiology of our responses to SARS-CoV-2. Differences in male and female immunological activity can be related to their differing vulnerability to the disease. Compared to males, females mount stronger immune responses to combat and clear viral loads.[29] The higher incidence of autoimmune and inflammatory diseases in women has long been known. With vaccines females can produce over-exuberant responses, of both the innate and adaptive immune systems, estimated to be twice as strong as in males. Sex-dependent steroid hormones and genes have been linked to the mechanism determining differences between the sexes in response to viral infection.[30] Around 60 genes associated with immune responses are present on the X chromosome. Despite inactivation of one copy, there is evidence for gene imbalance, favoring females, and their immunological responses to viral infection.[31] One example is Toll-like receptor 7. The gene for this receptor which senses RNA viruses such as SARS-CoV-2 is present on the X chromosome and may escape X cell inactivation.[32] Estrogen and progesterone receptors present on immune cells act as transcriptional regulators. During COVID-19, lung immune cells produce a "cytokine storm;" specifically, interleukin-6, interleukin-1β, and tumor necrosis factor-α, along with infiltration of chemokines, occur, resulting in lung injury and respiratory difficulties.[33] The protective effects of estrogen and progesterone have been attributed to their promotion of anti-inflammatory cytokines, increasing helper T and B cells and thereby antibodies. In addition, they suppress production of pro-inflammatory cytokines and migration of macrophages and monocytes into infected tissue.[28,34] Thus, we may expect that the immune landscape during a SARS-CoV-2 infection will look different between men and women and make the former more vulnerable to COVID-19.

Physiological studies of infection with SARS demonstrated female mice have lower viral loads, lower inflammatory responses, and reduced lung damage and death, compared to males; this protection was lost with ovariectomy or treatment with estrogen receptor antagonist.[28] For details of how women and men differ in their immune responses to COVID-19, the comprehensive study of Takahashi *et al.* is recommended.[35] A conclusion from their findings is that females could benefit more from therapies that dampened their innate immunity responses during initial infection period.

Disaggregating patient data by sex show key differences in the immune and physiological landscapes. This heterogeneity in immune capabilities and responses helps understanding of the distinct COVID-19 progression in women and men and may be used to guide disease prognosis and sex-specific treatments.[33,36] As males tend to have more severe COVID19, their enhanced inflammatory responses and higher B-cell recruitment, could make them more useful therapeutic plasma donors. The protective effects of estrogen and progesterone have stimulated novel treatment trials, involving, for example, anti-testosterone treatments or oestradiol patches being given to men.

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In summary, I hope I have conveyed how our physiological understanding of traditional sex differences, has been applied to non-traditional situations to help postmenopausal women and female-to-male individuals, have children. In addition, our growing understanding of non-traditional physiological sex differences has helped us understand, increased male vulnerability to infection with the SARS-CoV-2 virus, by mechanisms that include incomplete X chromosome inactivation of immune genes, a crucial role ACE2, and regulation of both immune activity and ACE2 by sex steroids.

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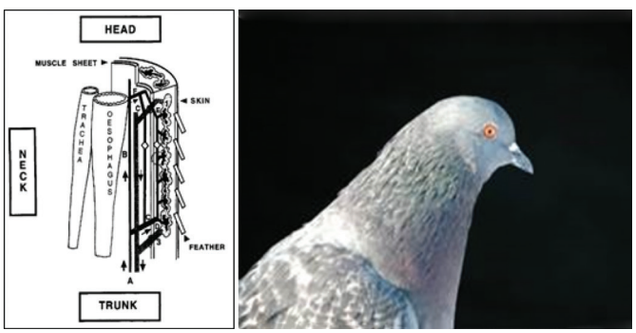
Fluffed posture of feathers i.e. ptiloerection works for birds, both in hot and in cold weather. Courtesy: Heikki Pönkkä and Esa Hohtola.

Almost all what a small bird needs in the Arctic: thermogenesis on breast muscle, fat stores for energy, feathers for insulation. Courtesy: Esa Hohtola.



Still a puzzle – the functional role of the collar plexus in thermoregulation in extreme environments. Courtesy: Liisa M Peltonen (adapted from Baumel et al. 1983) and C-M Peltonen. Courtesy: Liisa Peltonen, University of Helsinki, Helsinki, Finland.

Stretching the Limits – Comparative Physiology of Avian Thermoregulation in the Heat and Cold

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The Class *Aves* of the animal kingdom is species-rich and widespread. There are currently almost 11,000 known species in the world.[1] Birds' ability to fly is connected to endothermy, in combination with good thermal insulation by feathers, high efficiency of flying muscles and thereby, good aerobic capacity, and metabolic power.[2] The evolution of endothermy has been linked to selection for a high level of activity sustained by aerobic metabolism.[3,4] The evolution has progressed in three stages, starting from ectotherm ancestors in Permian – Triassic ages and reaching its final stage in Cretaceous and Late Cenozoic, at the time when birds began to fly, and climate started to cool. The triphasic model of evolution argues that tight endothermy was abandoned at any time that the bird did not rely on its thermal benefits to breeding success.[5] The physiological flexibility of birds manifests in their ontogeny, which involves a transformation of an ectothermic embryo to an endothermic adult.[6] Modern birds can switch to adaptive heterothermy to save energy and avoid predators in off-breeding seasons. Research during the past 20 years has shown that most birds are capable of short-term shallow torpor, especially when energetically challenged.[7] Birds also use facultative hyperthermia to save energy and water. Birds are extremely tolerant to high body temperatures (T_b)[8] and have a normal T_b 3–4 °C higher than other terrestrial animals of the same size. Based on heat transfer laws, this reduces the thermal gain from outside by reducing the temperature gradient between the body and the ambient air.

Comparative research of desert birds has become increasingly collaborative and researchers combine their data from arid zones around the globe. The trait of heat tolerance includes substantial phenotypical plasticity of metabolism and thermoregulatory mechanisms, and genetic variation due to natural selection.[9–15] Global climate change has challenged researchers to study responses to changes in ecosystems and the risks of sudden and extreme weather events that are characteristic of global warming.[16] The periodic heat waves are physiologically more problematic to desert birds than the steady increase in ambient temperature (T_a). Short-term climatic events have little effect on the general physiology of birds, which may lead to lethal dehydration due to errors in the trade-off between water conservation and thermal homeostasis.[17,18]

Having to face intensive solar radiation and T_a higher than T_b, diurnal desert birds rely on metabolically costly evaporative heat loss by respiration (REWL, panting), and to more economical and phylogenetically older vibration of the upper esophagus, driven by the hyoid bone (gular flutter). The gular flutter accompanies panting in many species.[19,20] The simultaneous activation of these mechanisms increases the efficiency of heat loss and reduces the energy cost. Moreover, the risk of respiratory alkalosis during combined evaporative heat loss becomes smaller when the exhaled air is mostly water vapor.[14]

It was formerly thought that the plumage and lack of sweat glands prevent any significant water loss from the avian skin. Since the pioneering studies of cutaneous evaporative water loss (CEWL) in the 1980s,[21–23] CEWL has been shown to be an alternative and highly efficient mechanism of heat dissipation. In the motionless incubating parent, CEWL contributes to maintenance of a low basic metabolic rate, thermoneutral T_b and adequate body-to-brain temperature

difference, all of which protect the eggs from overheating and ultimately contribute to breeding success.[24-26] So far, the ability to use flexibly various evaporative heat loss mechanisms has been restricted to orders Passeriformes, Columbiformes, and Caprimulgiformes.[10,13-15,23,27]

The biology of the key element of CWEL, the avian skin, is largely unclear. However, in danger of dehydration the facultative waterproofing of the epidermal tissue may happen rapidly.[28] In heat-acclimated Rock pigeons, α_2- and β-adrenergic receptors are involved in the initiation of CEWL in the central nervous system, as well as in the cardiovascular system by increasing the cardiac performance and permeability of cutaneous blood vessels.[29,30] In pigeons, acclimation to cold, hot and mesic environment modifies skin structure and affects the cutaneous blood flow responses to nitric oxide, indicating adaptive changes in the cutaneous circulation and the function of the epidermal water permeability barrier.[31,32]

Birds are very cold-resistant because of their high metabolic capacity and effective insulation. All birds rely on shivering thermogenesis to increase their heat production in cold.[33] Stimulated by the recent advances in the study of non-shivering thermogenesis in brown and beige adipose tissue of mammals, several claims on the existence of a similar mechanism in birds has been emerged. However, birds lack the gene for uncoupling protein 1, which is the basis for all mammalian non-shivering thermogenesis. As yet, solid evidence of shivering-independent adaptive thermogenesis (i.e. heat production activated for cold defense and regulated minute-by-minute according to thermal needs) has not been published. Avian shivering does not incur visible tremors, but is a smooth contraction produced by randomly twitching skeletal muscle fibers.[34] Thus, EMG recordings showing the absence of shivering are a prerequisite for any study claiming a non-shivering route for cold-induced thermogenesis in birds. The biochemical changes in muscle upon cold-acclimation are very similar to exercise-induced modifications resulting from increased shivering.[35]

The capacity of long-term aerobic activity in avian muscles is high enough to allow for sufficient thermogenesis even without seasonal changes in muscle mass,[36] although pectoral muscle mass correlates with summit cold-induced metabolic rate.[35] This enables many small passerines to survive the winter in high latitudes without seasonal migration. Reduced activity and immunosuppression allow the allocation of energy to cold defense.[37] As wintertime fat stores of a typical passerine can sustain the birds only 1–2 days, small birds in extreme cold cannot rely on fat stores as larger birds, such as penguins and Svalbard ptarmigan. Furthermore, because of their mass-to-surface ratio, small birds have much greater relative thermal conductance compared to, for example, non-migratory owls and are thus more dependent on active thermogenesis. Special behavioral adaptations of some passerines (huddling, roosting under the snow cover, and food hoarding) are classical examples of adaptation to a harsh environment.

The future comparative research on avian physiology continues to be collaborative, interdisciplinary and global, focusing on ecosystems, and impacts of their changes on avifauna. Innovative methodology, such as thermal imaging and various bio-logging approaches will further increase our understanding of avian thermoregulatory mechanisms.[38-40] Finally, despite the evidence of substantial phenotypic flexibility of avian physiological variables, the anthropogenic climate change has already caused collapse in avian populations in arid areas around globe. At the present, the population trends of several species living in desert or dry savanna habitats are decreasing.[1,41-44]

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Ethics Committee's Efforts on Issues of Concern in the Practice of Ethics in Research and Teaching of Physiological Sciences

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In Brazil, the National Council for the control of Animal Experimentation (CONCEA) has enacted a law (Arouca Law 11794) to deal with the ethical and legal issues related to the use of laboratory animals by having a "Commission for Ethics in the use of Animals" (CEUA) to be set up in all institutions. Strong legislations are in place to enforce it and, additionally the challenges of promoting ethical practices in animal experimentation are being met with by the universities by organizing courses for training professionals and updating users about these concerns. In 2018, CONCEA held the III SYMPOSIUM CONCEA with the theme "10 years of the Law Arouca (Law 11794)" that was attended by members of CEUA from all over Brazil.

The Brazilian Society of Physiology (SBFIS) has actively promoted and supported activities along these lines and among these were organizing a session on scientific integrity during the its Congress (SBFIS 2019) where Prof Dr. Renata Mazaro e Costa (current coordinator of CONCEA) delivered a lecture on "Dilemmas & Perspectives of Animal Experimentation in Brazil."

The Federal University of São Paulo, where Prof de Angelis teaches has not only started a regular postgraduate course in the Department Psychobiology on "Ethical Principles in Scientific Research" but also an e-learning course in "Ethics in Animal Experimentation" Furthermore, from this year (2019) onward, the University has started to observe a Biomedical Awareness Day (BRAD).

Prof Buckley has pointed out the "The Australian and New Zealand Council for the Care of Animals in Research and Teaching" (ANZCCART) recognizes that in training programs of researchers – communication strategies and media upskilling needs to be included so that not only the outcome of research is communicated but also consideration for the welfare of animals is communicated.

Some of the key issues that are being currently addressed in Australia are the absence of detailed reporting of methods (including any refinements used) and animal numbers in manuscripts that are being submitted for publication. These also include omitting the process of allocating animals to groups and mentioning blinding of groups, if done, in the details of results.

Furthermore, there are proposals afoot for having an animal trial registry that is similar in concept to the clinical trials registry which would provide momentum to the idea of greater openness in animal experimentation.

Prof Andrea Čalkovská has conveyed that an important issue was brought up in the recent Joint meeting of The Slovak and Czech Physiological Societies. This concerns the hitherto overlooked sex bias of applying data from male subjects (animals as well as human) to females and vice versa. Despite legislation changes in the US grant agencies and European research program (Horizon 2020), this bias is still present in a majority of research disciplines since male subjects are preferred in preclinical research. However, when the results of basic research are translated into clinical practice to improve the quality of healthcare – they will impact women's health.

Since the opposite bias also exists especially when data obtained mainly from animal models utilizing female

subjects, for example, from research in multiple sclerosis, and osteoporosis, which will thus skew the outcome when applied to men affected by these diseases – it is necessary that experiments be performed on equal number of both sexes before any valid conclusions be made, unless of course the studies specifically address reproduction or sex-related behaviors

Prof Olatunji-Bell organized a workshop (2020) on behalf of The Physiology Society of Nigeria at The Lagos State University College of Medicine, Lagos to update the medical student and research fraternity of all Lagos medical schools including research scientists and technologists from other Institutes of Lagos about current practices in ensuring ethical conduct in animal and human (clinical) research studies. While it was feasible to reduce the number of animals in future protocols – discussions centered around the feasibility of a complete replacement, by alternate methods, if the nature of the study necessitated their use.

At their meeting the Slovak and Czech physiologists too pointed out that completely switching over to the 3 Rs approach in Physiology (Replacement, Reduction, and Refinement) in preference to holistic models had several limitations – eminent amongst which was that cell cultures studies do not fully show what happens in the whole body.

Future Meetings and Symposia

Prof Kitazawa has initiated the Research Ethics Committee of the Physiological Society of Japan to hold a symposium on "The ethics, laws, and guidelines for human and animal researches" during the upcoming Annual Meeting of the Japan Physiological Society (March 19, 2020).

The subjects discussed will be -

- *"The past, the present, and the future of the animal protection law" – Dr. Naoko Kagiyama*
- *"Research guidelines for the ape studies" – Dr. Katsuki Nakamura*
- *"Laws and guidelines of the research ethics in human studies" – Dr. Tsunakuni Ikka*
- *"Ethics of human imaging studies" – Dr. Norihiro Sadato.*

The Way Forward

At present, in Brazil, efforts are underway to move away from and replacement of animal experimentation (de Ávila RI, Valadares MC. Brazil moves toward the replacement of animal experimentation. Altern Lab Anim. 2019;47:71-81).

In Australia, too Animal Ethics Conferences have been discussing the appropriateness of continuing to use animal models and the need for greater (financial) investment in replacement systems (e.g., organ on a chip, computer simulation or working with other models such as using drosophila or zebra fish), or refinement of techniques to minimize distress to animals such as remote assessment, or including imaging techniques to reduce overall study numbers, has been made. Mention has been made of New Zealand making available a $50,000 grant to support developing ways to replace, reduce, or refine the use of animals in research, testing, and teaching (https://www.mpi.govt.nz/protection-and-response/animal-welfare/animals-in-research-testing-and-teaching/the-3rs/).

IUPS Physiome Project

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The IUPS Physiome Project[1] is developing a modeling framework to deal with multiscale biophysical processes that link the organ systems of the human body to molecular mechanisms through models of cells, tissues, and organs. A major part of the project has been the development of standards for encoding both models and data to ensure that the models are findable, reproducible, reusable, and modular.[2] Models of subcellular processes such as transcription, metabolism, signaling, electrophysiology, myofilament mechanics, cell growth, and cell division are encoded in a markup language called CellML, developed to ensure reproducibility of these models. We have also recently launched a new journal, *Physiome*[3] to encourage the publication of curated and annotated physiological models based on these standards. The journal is managed by a board that includes representatives from IUPS, the University of Auckland, the Wellcome Trust, the US National Institutes of Health (NIH) and Digital Science (a publishing company that is providing philanthropic support for the journal).

Knowledge about physiological processes at the molecular and cellular scale developed by biomedical researchers worldwide is encoded in CellML models that are deposited in the Physiome Model Repository (PMR).[4] This database currently contains about 1000 such models and covers all areas of biology. The database will continue to benefit from the combined efforts of computational modeling groups worldwide and is the mechanism for bringing international physiological research into the multiscale whole body models. The new *Physiome* journal will help regulate the quality of the models by providing curation, annotation and documentation of these models and by requiring demonstrated reproducibility.

The NIH-funded *SPARC* project[5] on the autonomic nervous system is an example of an international project that is developing integrative Physiome composite models that will contribute to these goals. Other NIH Common Fund projects (e.g., HuBMAP,[6] which is mapping the spatial location of cells throughout the body) are now also taking advantage of the Physiome/SPARC mapping infrastructure. These lumped parameter models are coupled with spatial continuum models at the level of functional tissue units (FTUs), before being incorporated into whole organ models and linked with physiological systems models at the scale of the whole body.

The primary steps in the multiscale modeling process being developed for the Physiome Project are listed below.

Curation of CellML Models

The models of molecular and cellular processes, based on experimental measurements that are created by modeling groups around the world, provide the link from the tissue and organ models down to the molecular and cellular processes that underpin normal physiological function, and contain the mechanisms whose failure underpins clinical pathologies. To ensure reproducibility of the models, we code them in CellML and annotate the parameters and variables with ontological terms that facilitate semantic linkage to experimental and clinical data and algorithmic incorporation of the models into higher level models.[1,2]

Publication of CellML Models

The *Physiome* journal[3] provides a citable, documented version of reproducible and reusable models and simulation experiments and makes use of the PMR.[4]

Creation of Composite CellML Models

The ability to build complex models is crucially dependent on a modular approach in which components can be tested

independently before being incorporated into a higher level model. CellML has a simple structure based upon connected components. These components are abstract concepts providing well-defined interfaces to other components, and encapsulate concepts by hiding details from other components.[2] Connections provide the means for sharing information by associating variables visible in the interface of one component with those in the interface of another component. Consistency is enforced by requiring that all variables be assigned appropriate physical units, the dimensions of which must match when variables are connected. Clearly defined interfaces enable encapsulation hierarchies, providing further mechanisms for information hiding and abstraction. Model reuse is facilitated by the import element, enabling new models to be constructed by combining existing models into model hierarchies.

Creation of Whole Cell Models

Composite "whole cell" models are being developed to include, for example, membrane ion currents (and action potential generation), intracellular calcium handling, electro-mechanical coupling through calcium transients, force generation by the myofilaments, pH control, metabolism, and intracellular signaling.

FTUs

The next step in the modeling hierarchy from molecular and cellular processes to tissue and organ function is the FTU. These are 3D models of the organization of the cells and extracellular matrix within a small region of tissue associated with a particular physiological function. We have developed this concept[7] and applied it both to primary FTUs (pFTUs) in which any two points are within diffusion distance, and to secondary FTUs (sFTUs). sFTUs collect pFTUs into structures such as nephrons in the kidney, acini in the lungs, sheets in the heart, lobules in the liver, and osteon in bone that capture the physiological function of the tissue. pFTUs are centered around a small advective channel, such as a capillary, in preparation for higher-order assemblies of pFTUs in sFTUs.[8]

Physiological function emerges from molecular and cellular biology in an sFTU as a consequence of 3D structure and cell type, and these units are replicated many times to provide the higher level function of an organ (the pumping function of the heart, the gas exchange function of the lungs, the filtering function of the kidneys, etc.). Most organs have only one parenchymal tissue type (the pancreas is an exception where separate sFTUs are needed for the endocrine and exocrine functions) and there are only a few cell types present in each sFTU. As we build scaffolds for all organs, we also build scaffolds for the sFTUs for each organ and develop algorithms that assemble the sFTUs into the appropriate material coordinate locations within the organ (together with the spatial fields that define the spatial variation of sFTU parameters).

Organ Scaffolds

The next step up in spatial scale from an FTU is the organ. We have developed tools for building organ scaffolds to provide 3D material coordinate systems for registering tissue data.[9-12] The scaffolds allow cross-species comparisons and can be fitted to geometric data to match the organ anatomy for an individual. The SPARC project has funded the development of scaffolds for the heart, lungs, bladder, and digestive system (esophagus, stomach, duodenum, small intestines, cecum, and colon) with specific applications to mouse, rat, cat, pig, and human. The scaffolds use high order (tricubic Hermite) element basis functions but are designed to allow lower order finite element meshes of any resolution to be exported for use in computational models. With new funding from the NZ Government, we are planning to complete the development of scaffolds for all organs in the mammalian body over the next 5 years.

Organ System Models

We also require integrated models of all organ systems. The vascular system model, for example, will provide the anatomical layout of the vascular system (arteries and veins) in the adult male and female bodies, a database of material parameters (vascular wall thickness, elastic parameters, etc.), a bond graph model of the pressure-flow equations, and tools for solving these equations in a web browser environment.[13]

Integrated Whole Body Models

To be able to place organs and organ systems inside a whole body model (for any mammalian species), we are developing an approach in which annotations of fiducial material points, lines and surfaces on the organ scaffolds are also registered at corresponding material points in the 3D coordinate system of the whole body model.[14] This will provide a mechanism for ensuring that the organs and organ systems have a clearly defined functional and anatomical relationship to one another and that we can generate whole body models for any mammalian species and can generate statistical variation for a particular species.

A key step in multiscale modeling is having the ability to perform "model reduction" using AI techniques which work on large datasets created by running the computational models many hundreds of times under perturbations of the parameters and boundary conditions. We have substantial experience in this approach[15] and are now producing physiological models at the whole body level that are included in clinical workflows.

Physiology is characterized by systems that ensure homeostasis coupled with robust adaptive responses to environmental changes. It is clear that most phenotypes at the physiological level are extraordinarily robust to the deletion or down-regulation of individual genes.[16] Physiological function is therefore dependent on highly redundant networks that provide hierarchical control via multiple pathways at multiple levels of spatial organization. Feedback control exists at multiple levels within cellular networks, but it also exists at higher scales such as via the autonomic nervous system. A major focus for the Physiome Project in coming years will therefore be developing control systems models based on bond graph and port-Hamiltonian theory to represent these feedback loops and ensure that physical entities such as mass, charge, and energy are conserved.

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IUPS-BGA Questionnaire for all IUPS Adhering Bodies, Supporting Societies, Regional Members, Associate Members, Affiliated Societies for inputs[1]

[Questionnaire sent to Executive Director, President, General Secretary, Finance Director, and/or any other responsible executive members of the Organization]

1.0 Background Information:

1.1 Name of the Organization[2]:

1.2 Position of the signatory in the Organization:

1.3 Category of membership of your Organization in the IUPS (tick the most appropriate box):

- O Adhering Body
- O Supporting Society
- O Regional Member
- O Associate Member
- O Affiliated Society
- O Other (Please mention)

1.4 Geographical location[3] of your Organization (Please select the correct one from the following list and mention the country name):

- O Africa
- O The Americas
- O Asia
- O Europe
- O European Union
- O Middle East
- O Oceania

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1.5 Total members and Membership Fee:

	Regular Members	Graduate Student Members	Undergraduate Student Members	Affiliate Members
Total number				
Membership Fee				

1.6 Briefly describe what you consider to be the major advantages of being a member of the IUPS.

2. Based on the Recommendations of the IUPS 2017 Report on Physiology –Current Trends and Future Challenges, please provide below an update of activities recently conducted and/or being planned to occur in advance of IUPS 2021.

2.1 Have you developed networks and working groups domestically to facilitate the exchange of knowledge and best practices in teaching and research? If so, has the IUPS been involved? If yes, please provide brief details in the text box below.

- ○ Yes
- ○ Yes, but IUPS not involved
- ○ Yes, but IUPS was involved

2.2 Have you developed academic resources to improve the teaching and learning of physiology to ensure graduates have a full appreciation of the complexities of physiological understanding? If yes, please provide brief details in the text box below.

- ○ No
- ○ Yes

2.3 Have you developed an Outreach Programme to increase support among physiologists for furthering IUPS Initiatives and/or the World Health Organization's Health for All agenda? If yes, please provide brief details in the text box below.

- ○ No
- ○ Yes

2.4 Have you engaged in the development of a Global Mentorship Platform to facilitate Mentor/Mentee relationships among physiologists at various career stages, and in academic and clinical settings, to promote dialogue and aid career development? If yes, please provide brief details in the text box below.

- ○ No
- ○ Yes

2.5 Do you receive funding from government and/or private sources to aid the development of new initiatives designed to strengthen physiology as a discipline? If yes, please provide brief details in the text box below.

- ○ No
- ○ Yes

2.6 Have you implemented outreach activities to raise awareness of, and interest in Physiology among the lay public? If yes, please provide brief details in the text box below.

- ○ No
- ○ Yes

3.0. IUPS announced that the physiome is a new area for this millennium; however, this subject may not be fully acknowledged in some countries. Recently the scope of health management has shifted from the clinic to individualized general health care because of the development of many physiological signal-based devices including wearable devices. At this stage, physiology should go further to facilitate the development of new devices based on new physiological indices for the individualized general health care. Please respond below to explain how physiological data can be used to inform the development of new devices.

3.1 How can physiologists develop and encourage model (physiome)-driven physiological research?

3.2 Identify future research directions of physiological sciences in the era of data driven science including artificial intelligence.

3.3 What is need to be done in physiological science in the era of individualized health care?

4.0. Opportunities for the Development of Technical Skills for Learning and Research in Physiological Sciences
[Write briefly how your Organization considers collaboration with the IUPS (despite funding limitation) to support this venture]

5.0. OWhat are the Career Options for physiologists in your region?
Circle or place a checkmark in appropriate boxes.

- ☐ Researcher in Academia
- ☐ Teacher
- ☐ Researcher in Industry
- ☐ Academic administration
- ☐ Industry administration
- ☐ Science advocacy
- ☐ Science consultant
- ☐ Science writer
- ☐ Government
- ☐ Medical devices

6.0. Briefly describe the involvement of Organization(s) other than your own in the pursuance of learning, research skills and career strategies in physiological and life sciences in your region.

7.0. Briefly describe any new directions that are being undertaken in your region for translational medicine, regenerative medicine, and development of medical devices by the application of basic research to clinical medicine.

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8.0. Recognition of physiology education and research by Engineering Institutions to permit recruitment of physiologists in Biomedical Engineering in your region.

9.0. Provide suggestions for what the IUPS can do to help strengthen Physiological Sciences globally.

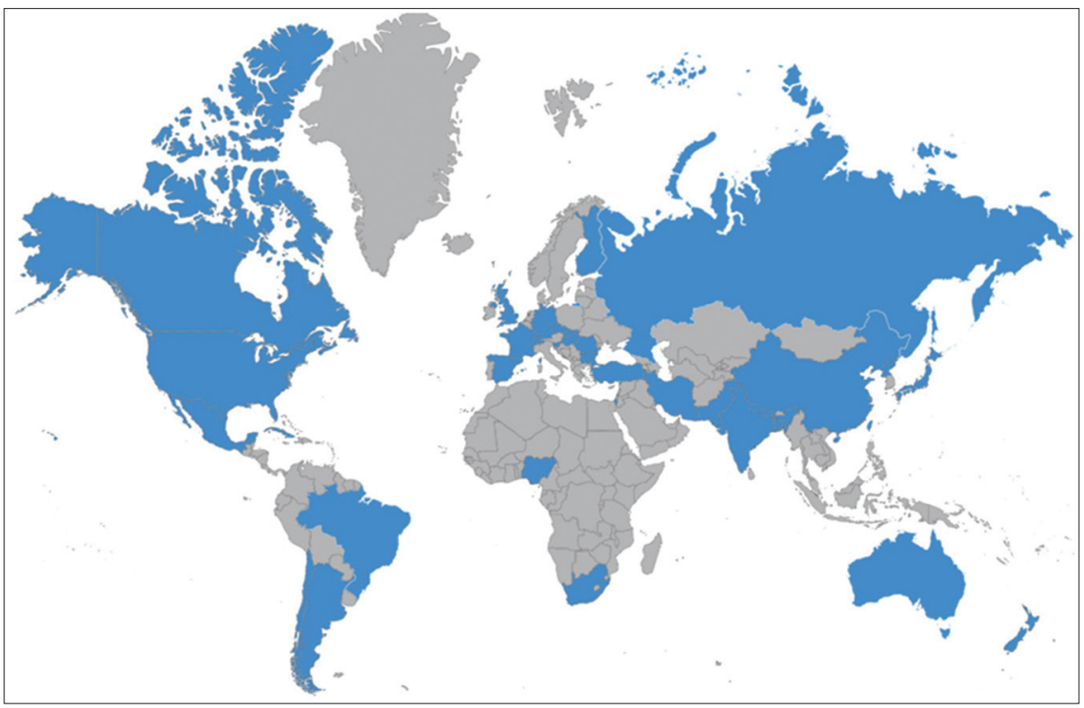
10.0. Add any other issue (s) your Organization would like the IUPS to address.

Signature:	
Name:	
Organization:	
Contact No.:	
Place:	
Date:	

IUPS, International Union of Physiological Sciences; BGA, Board of General Assembly; [1]For analysis and incorporation into the IUPS-BGA Document to be presented to the General Assembly in IUPS 2021 Congress; [2]Organization means Physiological Association, Society, National Committee, any Affiliated group of Physiologists; 3Based on the UN classification.

IUPS and its Member Societies across the Globe Contributed to the Report

Argentina, Australia, Bangladesh, Brazil, Bulgaria, Canada, Chile, China, Cuba, Czech Republic, Finland, France, Germany, Hungary, India, Israel, Iran, Japan, Korea, Mexico, Nepal, New Zealand, Nigeria, Pakistan, Romania, Russia, South Africa, Slovakia, Slovenia, Spain, Switzerland, Turkey, Taiwan (Chinese Taipei), United Kingdom, United States of America



Physiology – Challenges and the Way Forward

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Susan M. Barman is the Vice Chair of the Board of the General Assembly of the IUPS. Susan M. Barman, PhD, is a University Distinguished Professor, Department of Pharmacology & Toxicology and the Neuroscience Program, College of Human Medicine at Michigan State University. East Lansing MI USA. She has been very active in the leadership of the American Physiological Society, including serving as its 85th President. She has been a strong advocate of developing programs that promote the discipline of physiology and that encourage trainees to be actively engaged in the society. In addition to being an author on over 100 research articles and invited reviews on neural control of the circulation, she is a co-author of Ganong's Review of Medical Physiology, which is read by medical students in many countries.

The IUPS is the non-profit global umbrella organization for physiology, representing and promoting the worldwide community of professional physiological scientists and educators. Since its founding in 1953, IUPS has aimed to facilitate initiatives that strengthen the discipline. IUPS is a scientific union member of the International Council for Science and is accredited with the World Health Organization (WHO). The Union is composed of 44 Adhering Bodies, 14 Supporting Societies, 13 Associate Members, 5 Regional Federations, and 2 Affiliated Societies

Project Development, Research, and Analysis

- *Jayasree Sengupta, Chair, Board of the General Assembly, IUPS*
- *Susan M. Barman, Vice Chair, Board of the General Assembly, IUPS*
- *Members of the Board of General Assembly, IUPS*

Writing

- *Jayasree Sengupta, Chair, Board of the General Assembly, IUPS*
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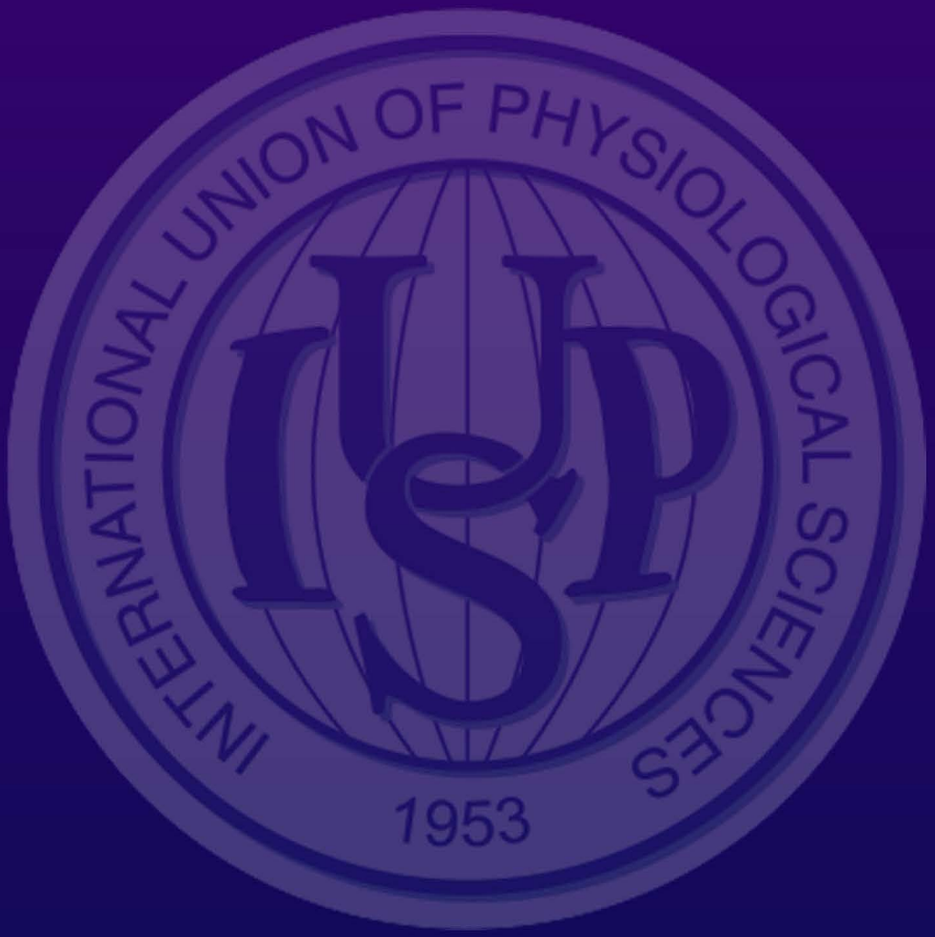

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