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# FROM THE EDITOR

## A new man at the ISH helm, who will make ISH flourish again!

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Editor

Dear reader,

It is again a pleasure for me to present a new double issue of Hypertension News (Opera 71–72) to you. Hypertension News is the web-based Newsletter of the International Society of Hypertension (ISH) and has been its main source of news and scientific exchange for about 20 years.

A month ago, and after some very difficult years, the members of the ISH and other hypertension researchers were finally able to meet again in Kyoto in Japan. About 3,000 participated, half of them in person. The Japanese hosts, under the leadership of Hiroshi Itoh and Hiromi Rakugi, should be commended for arranging a very good meeting in beautiful surroundings, having had major difficulties in getting us there. In this issue of Hypertension News (pages 4–19), you will find a series of reports from that meeting, including one from the organisers, one from Victor Dzau (pages 6–8) and one from Suzanne Oparil (pages 10–13).

At the end of the ISH meeting in Kyoto, Bryan Williams from London, UK, took over as the new President of the Society. With his outstanding scientific merits in cardiovascular medicine, his broad knowledge of recommendations and guidelines, and his good common-sense, Bryan Williams has every chance to make the ISH flourish again. We bid him welcome and wish him the best of luck with his new undertaking!

To present Bryan Williams to you, I have asked my old friend, Stuart Spencer, who is a senior executive editor at *The Lancet* and an honorary member of the ISH, to interview him (page 2). Over the years, I have not often seen Stuart enthusiastic, but this is how he starts his interview on page 2, "Wow! I had a conversation with our new president of ISH and came away stimulated and excited. It was supposed to be an interview but, from the first reply to my opening greeting I had to do little except listen while Bryan enthusiastically outlined his vision for the society and for hypertension." Not everyone will agree with Bryan Williams's thoughts and intentions. They are certainly more radical than we have seen in the past, but, as Stuart Spencer puts it: "If half of them are successfully introduced the International Society for Hypertension could encourage radical changes that will benefit millions of people." I strongly recommend you read the interview and consider what it implies!

Our heavily read "Learning the Ropes" feature, first introduced in March 2019, has allowed for some of the most distinguished leaders in the hypertension field to introduce key topics in hypertension research and management to our readership. In this issue, the title of this section is: From bench to clinic: Nitrates in vascular biology. Several authors, who have significantly contributed to NO/nitrate/nitrite research, contribute an introduction and four state-of-the-art articles (pages 28–45) on their findings and thoughts in this intriguing field. Texts well worth reading! Sincere thanks to Thomas Unger for editing this section.

Moreover, in this issue there are three important comments on the TIME study, recently published in *The Lancet* (pages 20–27), where the authors showed that it didn't really matter if you took

Continued on next page.

your blood pressure lowering medication in the morning or in the evening. There is also a lovely presentation of Michael Bader's institute in Berlin, Germany, on page 46–48.

Finally, the next meeting of the ISH will be held on 19–22 September 2024 in Cartagena, Colombia, one of the most beautiful colonial cities in Latin America. Cartagena was declared a World Heritage site in 1984, due to its amazing architecture and history. In this issue, the president of that meeting, Patricio Lopez Jaramillo, gives us a first presentation of the planning (pages 50–53). Time flies, so please note the dates and reserve the funding – the meeting is only 22 months away!

To finish, let me thank my brilliant deputy editor Dylan Burger, the excellent ISH Secretariat, and all the members of the editorial team for their endless support and help! Thanks also to all the authors for their valuable contributions.

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# Interview with Bryan Williams

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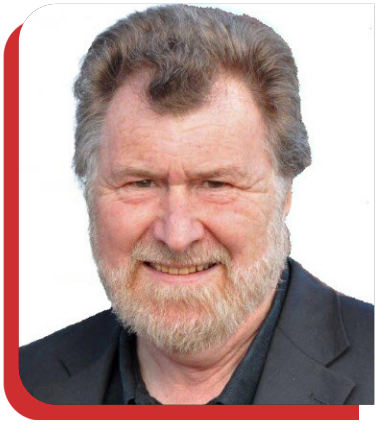

Wow! I had a conversation with our new president of ISH and came away stimulated and excited. It was supposed to be an interview but, from the first reply to my opening greeting I had to do little except listen while Bryan enthusiastically outlined his vision for the society and for hypertension. I did get in a few questions.

On the question of guidelines, Bryan was forward thinking. While accepting that European and North American societies (and their journals) benefit from producing their own guidelines, there was a case for low and middle income countries to develop their own guidelines. On the other hand, there is little difference between the various guidelines. Furthermore, he thought guidelines are not very effective in delivering improvements in the detection or, treatment and control of blood pressure; there has been too little focus on effective implementation in individual patients. Moving the focus away from the medical profession, and better empowering patients with information about what should be happening with them. To take responsibility for their own blood pressure would be in line with modern thinking and be more effective. This is something he wants ISH to do. He accepted that there would be resistance to this, but patient well-being should take precedence over vested interests. Given the very low price of common anti-hypertensive drugs, and many decades of safety data, he suggests there is a strong case for deregulating availability of these drugs. In response to the usual arguments against such an approach he had logical counter arguments. This also ties in with helping to make blood pressure control a greater priority for Governments. Politicians respond to public pressure from their constituents, so patient power can be more effective than pressure from industry and organisations. This is especially so when economic arguments showing the long term cost benefit of investing in blood pressure control, thereby reducing kidney and heart failure cases, are emphasised.

Similar thoughts were expressed in relation to low and middle income countries. Tackling the world's biggest killer, but it might be better achieved through using non-specialist platforms and by platforms to improve health care profession,

education and training. Aligning ISH members with predominantly non-physician experts in new technologies might aim to improve modern approaches to BP monitoring, such as smart wristbands and watches, with greater transparency on how they work. It might not be necessary to know blood pressure to 1 mm Hg when screening, but the value of such devices could be that the patient becomes aware they might be hypertensive and then refers themselves for assessment at an earlier stage. A new group within ISH could work constructively with technical partners to vastly improve detection and control of high blood pressure through these new approaches

Bryan also opined about the effect of current political movements on the future of ISH. An important part of the ISH strategy relates to education through meetings. The COVID pandemic, the war in Ukraine, and the ever-present concerns over climate change all impacted on attendance at the congress in Kyoto. However, Bryan reported that there was general consensus that face-to-face meetings are essential for advancing research and education and he was optimistic that face-to-face meetings will continue to be important for ISH. Hybrid meetings, he believes, can still allow face-to-face meetings and engagement. He also accepted that ISH has been dominated by high income countries, but that the future of ISH requires broadening the participation of all regions. Engagement with, and by, members in low and middle income countries needs to be encouraged and this might be achieved by increasing local or regional hybrid meetings. He also has thoughts on other ways that greater involvement of people in these regions can be encouraged.

Not everyone will agree with all of Bryan's thoughts and intentions. They are more radical than we have seen in the past but if half of them are successfully introduced the International Society for Hypertension could encourage radical changes that will benefit millions of people. Sit back and enjoy the ride.

Stuart Spencer - stuart.spencer@lancet.com

# THE 2022 ISH MEETING IN KYOTO

## Introduction

AKIRA NISHIYAMA

Department of Pharmacology, Faculty of Medicine, Kagawa University, Kagawa, Japan

Dear ISH members,

We are pleased to report that the 29th scientific meeting of the International Society of Hypertension (ISH2022 KYOTO) was held in Kyoto, Japan, with great success.

I am Dr. Akira Nishiyama who was in charge of the PR committee of the Local Organizing Committee (LOC) of ISH 2022 KYOTO. Here is my report on ISH 2022 KYOTO.

Since 2016, when it was decided that we, the Japanese Society of Hypertension (JSH) are to host ISH2022, the LOC members of JSH have made every effort as a team to make the meeting productive for all participants. However, the biggest unseen enemy was COVID-19. In 2022, COVID-19 continued to rage around the world. As a result, the action restrictions had not been lifted in many parts of the world. In particular, entry restrictions to Japan were lifted on October 11, just the day before the ISH was held. Therefore, LOC members were concerned that there would be very few local participants in ISH 2022.

His Imperial Highness Crown Prince Akishino giving his opening address.

Thus, ISH2022KYOTO was facing great difficulties right up to the last minute. However, about half of the approximately 3,000 participants were on-site participants from 67 countries. In addition, we have received many messages from participants impressed by sophisticated and excellent lectures with variety and heated and productive discussions performed by you.

The opening ceremony was held with the participation of Their Imperial Highnesses Crown Prince and Crown Princess Akishino (see photo). The fact that His Imperial Highness Prince Akishino made a strong appeal for the importance of hypertension research at the opening ceremony on the first day of the event was a major topic of discussion, as it was extremely unusual.

Special lecture by Prof. Victor J. Dzau on the new Hypertension was followed by four days of special lectures by eight top leaders, as well as numerous symposiums and other events. The topics ranged from regenerative medicine by Nobel laureate Prof. Shinya Yamanaka to Astronauts, Zen, Robots, Food, Sleep, and other areas important to the future of hypertension. In addition, oral and poster presentations were also heated and showed the excitement that can only come from an onsite event. We believe that you found something worthwhile and someone valuable for your science and medical practice of hypertension. We had a very exciting program on all aspects of blood pressure-related science, and we received many compliments from the attendees on what an excellent program it was.

At the gala dinner, the new ISH council members, who were chosen through elections, were announced (see the ISH announcement for more details). Then there was a scene where all the old and new ISH executive council members were congratulated by Ninja.


On-site participants surely re-discovered the wonderfulness and joy of face to face communication with your old and new friends. At the closing ceremony, ISH2022KYOTO Hypertension Zero Declaration was presented.


Then, Colombia, the next ISH scientific meeting place, was introduced and participants pledged to meet again. Thus, ISH2022KYOTO proved to be a great success.

We appreciate your contribution again for making this meeting successful. I hope this meeting will lead to productive collaborations with researchers in the world. By the lessons you learned and with the colleagues you found in this meeting, I hope that you continue to seek for the wisdom for conquering hypertension.

I sincerely hope for world peace. See you soon in Colombia!

Sincerely yours,

From all JSH LOC members

Akira Nishiyama - nishiyama.akira@kagawa-u.ac.jp

# THE 2022 ISH MEETING IN KYOTO

## Hypertension: past successes, present challenges, and future promise

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Despite the fact that hypertension is a major risk factor for cardiovascular disease, it remains a condition without an effective strategy for global control at the population level. Significant progress has been made in the development of increasingly effective drug treatments; however, hypertension detection, awareness, and control remain problematic worldwide.

Major advancements are needed to transform the field of hypertension. My colleagues and I have previously addressed the need for major advances in biomedical, digital, and data sciences, as well as an international, population-based strategy.[1] This paper will review where the field has been, the challenges facing us today, and the possibilities for future directions.

**History of Hypertension and Past Success**

Almost 5,000 years ago, The Yellow Emperor's Classic of Medicine stated, "...(h)ence, if too much salt is used for food, the pulse hardens...." William Harvey is credited as the first in the Western world to describe circulation in the 1600s, while Stephen Hales is known as the first to directly measure blood pressure in living being – a horse – via his invention of the manometer. In the 19th century, British physician Frederick Akbar Mahmoud worked to update the sphygmograph device, used to measure arterial blood pressure in humans, and later published his findings in the measurement of blood pressure in patients with Bright's disease.[2] Ultimately, this work led him to the observation that high blood pressure was linked to kidney disease.

Seventy years ago, the Framingham study was instrumental in documenting that hypertension is a major risk factor for cardiovascular disease.[3] Findings from the study transformed the field of hypertension. Since then, we have seen major advances in hypertension research, notably, a more comprehensive understanding of the physiology and biochemistry of blood pressure regulation, studies of dysregulation as a basis for hypertension, the generation of animal models, and the development of effective medications.

Indeed, major advances have been made in the understanding of the mechanisms of blood pressure regulation, which includes the renin angiotensin aldosterone (RAS) system, the sympathetic adrenal axis, and endothelin; as well as nitric oxide, kinin, and atrial natriuretic peptide. Using RAS as an example, significant progress has been made in biochemistry, physiology, molecular, and clinical research which has led to elucidating its role in cardiovascular regulation and diseases, and development of effective inhibitors of the RAS. An important development in the long journey of RAS research has been entrance to the age of molecular and vascular biology. This has included the cloning of renin, which provided understanding of mechanisms of renin expression and tissue specific regulation, and knowledge of molecular structure enabling the development of pharmacologic inhibitors. The cloning and molecular characterization of

angiotensin receptors provided knowledge of angiotensin receptor structures, signaling, and function; the identification of angiotensin II receptor subtypes; and their counter-regulatory roles in cardiovascular regulation.[4]

Work in vascular remodeling and disease has been essential to the contemporary understanding of the role of RAS in hypertension and cardiovascular disease. Studies from my lab and others provided evidence of the existence and function of the tissue angiotensin system, in that angiotensin is produced locally in the heart and the blood vessels, and local angiotensin exerts direct actions on the local tissue.[5]

Work performed by Hiroshi Itoh and my lab was the first to demonstrate the effect of angiotensin II on vascular smooth muscle cell hypertrophy and proliferation in vitro. Using gene transfer techniques, Ruyichi Morishita confirmed the angiotensin's effect on vascular remodeling in vivo. Work in my lab by Hiromi Rakugi provided evidence for the activation of tissue angiotensin-converting enzyme (ACE) and angiotensin II in experimental vascular injury and human vascular lesions. Our data suggested that inflammatory cells can produce local angiotensin, which mediates vascular pathology. The clinical importance of this research has been demonstrated by the significant reduction of cardiovascular events in clinical trials of RAS inhibition. RAS inhibitors are lifesaving drugs used globally to treat hypertension, heart failure, and cardiovascular diseases.

**Future Promise in Research and Development**

Based on decades of research, there are many classes of effective drugs for hypertension treatment, including vasodilators, peripheral sympathetic inhibitors, monoamine oxidase inhibitors, diuretics, beta blockers, and ACE inhibitors. Newer classes of therapeutics are currently being developed, including endothelin receptor agonists, aldosterone synthase inhibitors, and SGLT2 antagonists, among others.

Recent biomedical advances provide exciting promises for the future – moving ultimately to prevention and cure. Indeed, these include (1) vaccines targeting renin, angiotensin II, and AT 1 receptors; (2) RNA interference or post-transcriptional gene silencing of angiotensinogen; and (3) gene editing. Germline gene editing offers the potential of cures for heritable monogenic hypertension. Importantly, recent data from our lab suggested that somatic gene editing may be effective in controlling or potentially preventing systematic hypertension. We demonstrated that using CRISPR/Cas9 gene editing in the angiotensinogen gene in the liver reduced blood pressure in SHR with established hypertension, and prevented the development of hypertension in young, prehypertensive SHR. Effects are long-lived, with blood pressure control observed over a year of follow up.[6] This represents a potential one-step treatment for human essential hypertension, with simple administration and without observable side effects.

**Meeting Challenges in Hypertension**

Still, significant challenges remain. Awareness, treatment, and control of hypertension are poor, particularly in low- and middle-income countries. We see difficulties achieving early detection of elevated blood pressure and loss of patients to follow up. Treatment is lifelong, relatively arbitrary, and imprecise. Frequent drug side effects lead to noncompliance. Considering blood pressure alone often misses the many influencing factors, including social, behavioral, and dietary variables, as well as the role of physical activity. Moreover, the pathophysiology and genetics of essential hypertension remain unclear.

Digital health represents an area ripe for innovation, allowing the potential for improved access to care, remote monitoring and connected care, improved patient adherence, integrated behavioral coaching, and the collection of data from new types of patient sensors and wearable technologies. There exists a major need for expansion of health data sets to include information on social determinants, community and environmental factors, and issues of access and equity. Hypertension management must be data driven and evidence-based, but current guidelines based on data from randomized controlled trials do not address diversity in patient lifestyles. Data integration across care delivery and public and community health is needed to achieve advances in precision medicine and artificial intelligence (AI) research.

Finally, we must reach for a population health approach to hypertension. This would require the support of monitoring systems to improve the efficacy of treatment, a need for policy and systems change – e.g., achieving universal health coverage to ensure access to high quality care, and transforming health care delivery to achieve integrated, coordinated, high-value care – and the introduction of convergence science. Population health requires the engagement of social, behavioral, economic, data, legal, and political science sectors, and must involve policy decisions and practice in all areas.[7]

**Vision of the Future**

A vision for hypertension treatment in 2030 should include improved science and technology – methods of digital health, data science, AI, and machine learning. It must also include data integration, considering not only clinical and point-of-care data, but social, environmental, and behavioral data sets. Finally, it must include a focus on policy, public health, and community health, to achieve desired population outcomes. We have achieved a great deal since the initial description of hypertension in The Yellow Emperor's Classic of Medicine, but the field is ready for a transformation, and we must be prepared to tackle these challenges head on.

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Melissa Laitner - MLaitner@nas.edu

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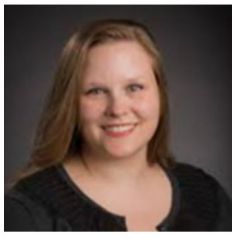

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# 2022 Franz Volhard Award International Society of Hypertension "My road to academic cardiology"

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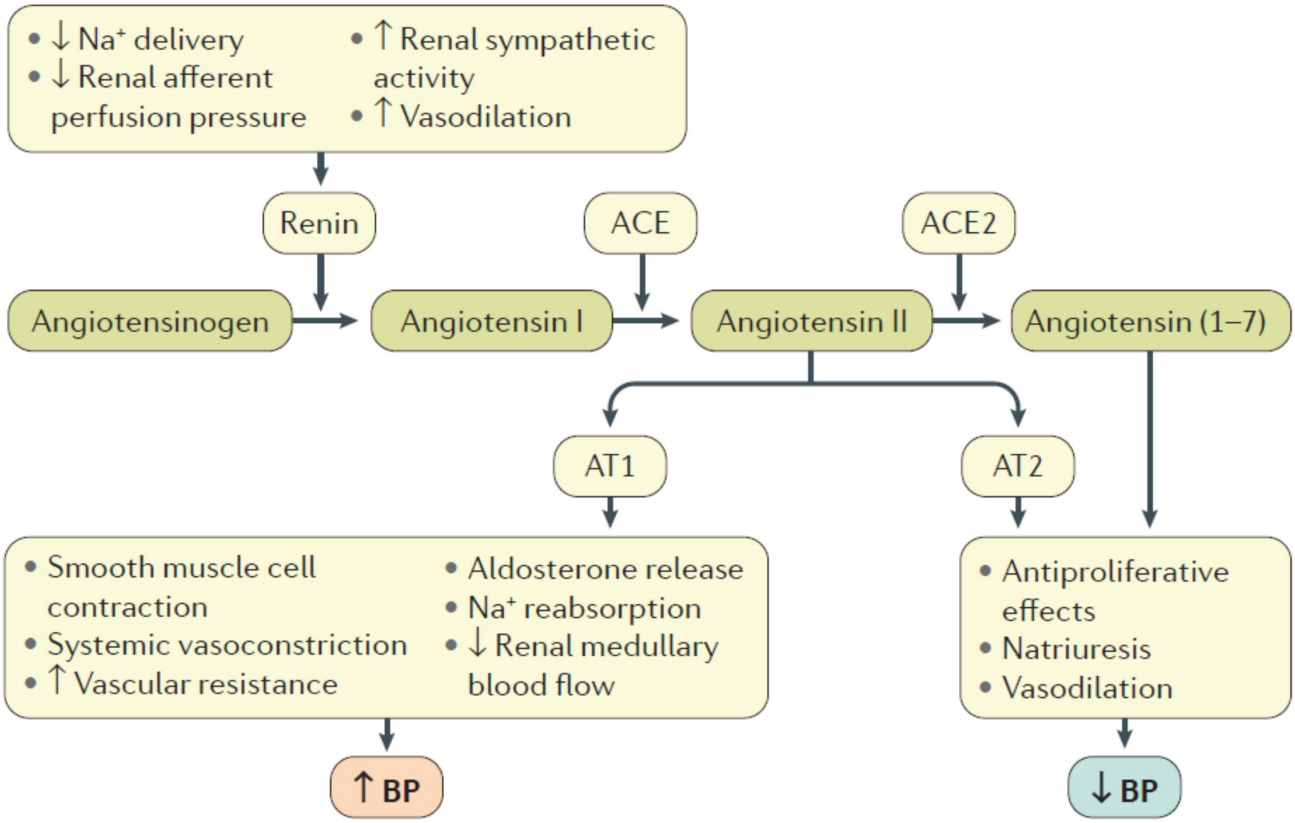
My road to academic cardiology started on our dairy farm in upstate New York, where I learned the pleasures and responsibilities of work at very young age. We were given a little lamb by a neighbor and I was given major responsibility as a young child for caring for him. The work ethic extended to schooling. I studied hard and completed undergraduate studies at Cornell and medical school at Columbia, then began medical residency at Columbia's teaching hospital. There my career was interrupted when the residency class ahead of me filled up with Vietnam veterans. That bad luck turned out to be the best of fortunes when I was introduced to Edgar Haber, then Chief of the Cardiac Unit at the Massachusetts General Hospital. Ed was an innovative scientist, a great mentor and a great leader in cardiovascular medicine. He spurred my interest for the first time in biomedical research and laboratory medicine. Under his guidance, I made a number of important observations concerning the role of the renin-angiotensin-aldosterone system in a variety of physiologic and pathophysiologic states (Figure 1). I was first to demonstrate that acute release of renin from the kidneys is required for adaptation to the upright posture in normal humans.[1] We measured plasma renin activity in normotensive volunteers, in patients who were undergoing diagnostic cardiac catheterization and patients who had their kidneys removed due to chronic kidney disease. We found that in the normal volunteers, plasma renin activity rose in response to upright tilting but that the renin release response lagged behind the increases in blood pressure (BP) and heart rate. We also found that a minority of the normotensive volunteers fainted when assuming the upright posture and that their increases in plasma renin activity were less than in those who had a normal BP response. Thus, we demonstrated for the first time that the renin angiotensin system functions abnormally in people with upright posture-induced hypotension and vasovagal syncope.

I was the first to observe, using radioimmunoassay techniques, that the major site of the conversion of angiotensin I to II is the lung.[2] My colleagues and I were the first to delineate substrate requirements for angiotensin I conversion to angiotensin II in vivo and in vitro.[3] These seminal mechanistic observations led to eventual development of the angiotensin-converting enzyme inhibitors (ACEI) as groundbreaking therapies for hypertension, heart failure and chronic kidney disease associated with diabetes and proteinuria.

My first translational research experience as a postdoctoral fellow was to examine the conversion of angiotensin I to angiotensin II by ACE in the pulmonary circulation under normal and pathologic conditions. This followed from my seminal observation that the primary site of conversion of angiotensin I to angiotensin II to supply the systemic circulation resides in the endothelial cells (ECs) of the pulmonary vasculature. I demonstrated that exposure to acute hypoxia was associated with rapidly reversible reduction in conversion of angiotensin I to II, indicating that acute hypoxia is associated with a reversible decrease in pulmonary ACE availability in the intact

**Figure 1. Role of renin-angiotensin-aldosterone system in BP regulation**




Decreased renal afferent arteriolar perfusion pressure, reduced sodium ($Na^+$) delivery to the macula densa (an area lining the wall of the distal convoluted tubule in contact with the glomerulus), activation of renal sympathetic nerves (via β1 adrenergic receptor stimulation) and a variety of vasodilators, including prostaglandin E2, stimulate the release of renin. Angiotensin II activates the type 1 angiotensin II receptor (AT1), triggering smooth muscle cell contraction, systemic vasoconstriction, increased renovascular resistance and decreased renal medullary blood flow, a mediator of salt sensitivity. Stimulation of the AT2 has opposite effects, resulting in vasodilation, natriuresis and antiproliferative actions. Cross-transplantation studies using wild-type mice and mice lacking the AT1 have shown that both systemic and renal actions of angiotensin II are relevant to physiological blood pressure (BP) regulation but that the detrimental effects of angiotensin II in hypertension are mediated mainly via the kidneys. Angiotensin-converting enzyme (ACE) inhibitors and AT1 antagonists have been shown to increase angiotensin (1–7) levels in the plasma and urine of normotensive animals and to enhance renal ACE2 activity. Studies in rodents and humans with diabetic kidney disease suggest that upregulation of ACE2 delays progression of kidney disease.

Reprinted with permission from Springer Nature, Nature Reviews Disease Primers. Oparil S, Acelajado MC, Bakris GL, Berlowitz DR, Cífková R, Dominiczak AF, Grassi G, Jordan J, Poulter NR, Rodgers A, Whelton PK. Hypertension. Nat Rev Dis Primers. Vol 4, Article No. 18014 (2018). doi:10.1038/ .2018.14.

dog.[4] I later demonstrated impaired pulmonary conversion of angiotensin I to angiotensin II in rats exposed to chronic hypoxia. In translation to the human condition, I examined ACE activity in plasma from normal humans and those with sarcoidosis, chronic obstructive pulmonary disease (COPD) and shock lung.[5] Major findings of the study were that patients with sarcoidosis had increased plasma ACE activity whether or not they were receiving steroid therapy, whereas those with COPD and shock lung had decreased ACE activity, revealing for the first time that plasma ACE activity is a reflection of pulmonary conversion of angiotensin I to II and can be altered by pulmonary disease. A subsequent study tested the hypothesis that measurement of serum ACE levels would be useful in differentiating cardiogenic pulmonary edema, in which pulmonary ECs would be expected to be intact, from ARDS, which is characterized by EC damage/dysfunction.[6] I found that serum ACE levels were markedly decreased in patients with ARDS and sepsis, while ACE levels in patients with cardiogenic pulmonary edema and those undergoing cardiopulmonary bypass were not different from healthy controls. My co-authors and I speculated that decreased ACE levels in the setting of sepsis and ARDS are due to the presence of circulating inhibitors of ACE. We concluded that the finding of decreased serum ACE can be of potential clinical usefulness by raising the possibility of sepsis as the etiology of ARDS before results of blood cultures are available.

My laboratory was the first to study the role of endothelin-1 (ET-1) and its receptors (ET-AR and ET-BR) in the development of hypoxia-induced pulmonary hypertension and vascular hypertrophy.[7] We demonstrated that hypoxic exposure stimulates ET-1, ET-AR and ET-BR mRNA and protein expression. The increase in ET system activity is the primary cause for the hypoxia-induced pulmonary vascular constriction and hypertension. Using gene promoter activity analysis, we found that the *EDN1* gene is a hypoxia-inducible gene in pulmonary arterial endothelial cells. We demonstrated that treatment with selective ET-AR blockers can prevent and reverse hypoxia-induced pulmonary arterial constriction and hypertension, as well as right ventricular (RV) hypertrophy. In contrast, we found that treatment with selective ET-BR blockers can exacerbate these ET-dependent processes. We have also helped several pharmaceutical companies to screen the potency and efficacy of the ET receptor antagonists that they generated. We identified bosentan as the most potent non-selective ET-AR and ET-BR blocker. Bosentan is currently being used to treat patients with pulmonary hypertension and cardiac failure.

Early in my career, I also developed an interest in hypertension and heart disease in women. Hypertension is the most common modifiable risk factor for cardiovascular disease, the leading cause of death in women worldwide. Young women are protected from developing hypertension, in part, by endogenous estrogen. As women age, they become more likely to develop hypertension and the associated cardiovascular disease outcomes. Women also have unique forms of hypertension associated with pregnancy, menopause, and the use of oral contraceptives. A specific issue that drew my attention was whether postmenopausal women should use hormone replacement therapy to prevent cardiovascular disease. At that time, postmenopausal women were recommended by the Women's Health Initiative (WHI) not to use estrogen replacement therapy for cardiovascular disease prevention because previous studies, carried out in older post-menopausal women, showed no benefit from estrogen. I hypothesized that the integrity of estrogen receptors and the vasoprotective effects of estrogen disappear with aging and tested the hypothesis that favorable responses to estrogen treatment are lost in injured arteries of aging animals (rats). I found that aged ovariectomized rats lost the vasoprotective and anti-inflammatory responses to exogenous estrogen that were seen in younger animals. These results may explain the lack of estrogen responsive in vasoprotection in the vasculature of elderly post-menopausal women treated with hormones.

My professional activities also include studies that bear on the care of patients with heart disease. I have for over 30 years played a leading role in the design, recruitment, and dissemination of results for large multi-center clinical trials of antihypertensive and cardiovascular therapies. I have been a member of Steering Committees of numerous important outcome trials in hypertension, including ALLHAT[8], LIFE, Simplicity HTN-3 and SPRINT.[9] Most recently, findings from

my studies in isolated cells and animal models have led to the design and conduct of a major trial that tested the effects of medical treatment of mild chronic hypertension in pregnant women.[10] CHAP demonstrated for the first time that in pregnant women with mild chronic hypertension, a strategy of targeting a BP < 140/90 mm Hg was associated with better pregnancy outcomes than a strategy of reserving treatment only for severe hypertension, with no increase in the risk of small-for-gestational-age birth weight.

None of this work could have been accomplished without the talents and efforts of my colleagues, trainees, and clinical research staff and the valuable funding provided by the NIH, AHA and other sponsoring agencies over the past 40+ years.

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# THE 2022 ISH MEETING IN KYOTO

## Clinical trials

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This year's ISH meeting in the beautiful city of Kyoto provided several sessions with interesting secondary analyses and long-term follow up data from some of the major clinical outcome trials in hypertension.

Starting early Thursday morning, there was a session on the association between blood pressure and dementia. Jeff Williamson from Wake Forest University, US, set the stage, reviewing some of the recent publications from the SPRINT-MIND study. In 2019, the SPRINT group found that intensive blood pressure-lowering was associated with a lower risk of the composite outcome probable dementia and cognitive impairment.[1] Although falling slightly short in their primary outcome analysis of probable dementia, their findings were recently supported by an individual-patient data meta-analysis of prospective cohort studies, showing reduced risk of incident dementia and Alzheimer´s disease with BP lowering in people with hypertension.[2] In Kyoto, Ruth Peters from Imperial College London, UK, presented results from a collaborative individual participant data meta-analysis of five randomized controlled trials; HYVET, ADVANCE, Syst-Eur, SHEP and PROGRESS. More than 28 000 participants, with mean age 69 years and baseline blood pressure 156/83 mmHg, were followed for an average of 4.2 years with a 10/4 mm Hg mean blood pressure difference between groups during follow-up. Including more than 800 cases of dementia, they found a significant 13% relative risk reduction in those randomized to more intensive treatment versus control. Reassuringly, no significant U-shape was found in relation to follow-up blood pressure for either treatment group. With these findings it seems reasonable to add dementia to the list of diseases not only associated with hypertension, but also preventable through blood pressure control.

During the late-breaking session, Dr George Siopis from Deakin University, Australia, presented a thorough systematic review and meta-analysis on the effect of digital interventions for people with hypertension. With dismal treatment and control rates across the globe, and particularly in low- and middle-income countries,[3] heads are now turning towards digital health for possible assistance. In conclusion, web-, app- and text message-based reminders for blood pressure recording and medication intake reduced blood pressure by an average of 3/2 mmHg with no significant difference between strategies. Dr Siopis concluded that text-based interventions have the largest potential because more than 90% of the worlds adult population have access to a mobile phone. Interestingly, the risk of bias across trials was generally rated as high, which is often true for small academic studies with blood pressure levels as key outcome. This observation emphasizes the need for more adequately powered, well-conducted trials addressing questions like monitoring and adherence.

Ending the late-breaking session, Peter Sever from Imperial College London, UK, presented long-term follow-up data from the ASCOT Legacy Cohort. In ASCOT, participants were randomized to either amlodipine with the possible addition of perindopril, or atenolol with the addition of bendroflumethiazide. Although the blood pressure difference between treatment arms was modest, the amlodipine group had 23% fewer strokes and lower all-cause mortality during follow-up.[4] Subsequent analyses have found that the observed differences in outcomes may be explained by differences in visit-to-visit blood

pressure variability between treatment groups.[5] During the meeting, Peter Sever presented 20-year follow-up data, suggesting that blood pressure variability, as opposed to mean blood pressure level, during the active phase of the trial predicts clinical outcomes during long-term follow-up. Although these findings are observational, and may reflect that visit-to-visit variability is a more refined way of assessing vascular health compared to mean blood pressure levels, it is intriguing to think that it may also be a potential therapeutic target to modify vascular ageing. Indeed, previous studies have come to slightly different conclusions regarding potential legacy effects of blood pressure-lowering,[6,7] whereas they have been more positive for statin trials.[8] Perhaps, the observed differences between hypertension trials reflect to what extent treatment have affected visit-to-visit variability rather than mean blood pressure itself?

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# Pediatrics: shifting the focus for preventing hypertension

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Primary hypertension is now more commonly observed in children and adolescents[1] than secondary hypertension. Several studies were recently developed to shift the focus of understanding the etiology of primary hypertension in children and adolescents, which sparked interest in many countries and stimulated large collaborative networks. At the past 29th Scientific Meeting of the International Society of Hypertension held in Kyoto, Japan, pediatric hypertension received more airtime at the conference than before, which highlights the importance of focusing on preventing hypertension from early life. Hypertension and cardiovascular disease originates in utero[2], known as early life programming, which is a critical period for fetal development. Complications during pregnancy as well as maternal lifestyle and family history of parents[3], can shift the life course of a developing fetus from an ideal life course trajectory to early vascular aging.

Joseph Flynn, a leading expert in the field of pediatric hypertension, shared his insights from the SHIP-AHOY study and explaining why a lower blood pressure threshold is of concern for children and adolescents.[4] With data supporting increased left ventricular mass and left ventricular hypertrophy at blood pressure levels as low as the 75th percentile[5], children can experience adverse effects of blood pressure at levels considered normal in the current clinical practice guidelines. The meeting was further enriched by presentations focusing on challenges and gaps in the management of hypertension in children and adolescents, which included discussions on accuracy, availability, validation and calibration issues of blood pressure devices used in the pediatric landscape. In addition, LMICs and others have limited access to pediatric validated devices, the correct cuff sizes and are faced with many challenges that compromise the management and proper diagnosis of hypertension in children.

The variety of hypertension guidelines in pediatrics was also discussed as a potential pitfall to countries that do not have their own nomograms for blood pressure. Such countries rely on reference values and blood pressure targets developed in countries with less ethnic diversity and divergent geographical backgrounds, supporting the efforts to sensitize the need for region-specific normative data, but to forge globalized guidelines for the management of hypertension in pediatrics. Lifestyle, environment, migration, sociocultural and psychosocial risk factors impact on hypertension development in childhood and adolescence. Currently the main driving forces for elevated blood pressure in the young is overweight and obesity, but data from the ExAMIN Youth SA study[6] has indicated that unique sex differences exist in the benefit of physical activity on the adverse association between excess adiposity and pulse wave velocity. Increasing cardiorespiratory fitness seems to be protective for arterial stiffness development in boys, but absent for girls. More attention is shifting to risk factors that distinctively associate with sex/gender and ethnicity/race to increase our understanding of pathophysiological mechanisms that will improve current treatment strategies and future preventive care.

With several unanswered questions, challenges in pediatric health care and the limited understanding of childhood onset hypertension, there is promise for exciting frontiers to explore and findings to present at the 30th Scientific Meeting of the International Society of Hypertension in Cartagena, Colombia.

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# THE 2022 ISH MEETING IN KYOTO

## Nocturnal hypertension

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During the Kyoto Scientific Meeting, nocturnal hypertension has been recognized as a significant risk factor for cardio and cerebrovascular diseases.[1] Blood pressure (BP) monitoring significantly increased our awareness of nocturnal hypertension. Dr. Parati and Dr. Kario highlighted the use of 24-h ambulatory blood pressure monitoring (24-h ABPM) as an important tool in nocturnal hypertension diagnosis, despite of its challenges (poor reproducibility of nocturnal BP levels and dipping status, sleep disruption, etc.).

New perspectives for nocturnal blood pressure measurements were also shown. The increasing use of home blood pressure[2] and portable devices[3,4] allows us to better understand the behavior of blood pressure. Although most of these new devices are under validation[5], they will open new lines of research such as comparison with 24-h ABPM, improvement of technical aspects of measurements devices, measurement schedule and conditions, prognosis impact, diagnostic usefulness and, maybe, new thresholds for the treatment.

Therapeutic approaches was another important issue discussed at the meeting. Dr. MacDonald presented the results of TIME Study[6] in which there were no differences between morning and evening doses in both primary and secondary endpoints. It seems that chronotherapy provided the best results only for special populations, potentially patients with isolated nocturnal hypertension.

There are studies which have demonstrated that some groups of antihypertensive medications are more effective in regulation of nocturnal BP. Regarding this topic, Dr. Kario presented not only the use of Esaxerenone in combination with RAS inhibitors and calcium channel blockers to treat nocturnal hypertension in uncontrolled hypertensive patients[7] but also, the long-term reduction in blood pressure after renal denervation in resistant hypertensive patients.[8]

Findings presented during the 2022 ISH meeting showed important advances regarding nocturnal hypertension. However, further studies are needed to achieve more accurate diagnosis and treatment, to finally improve the cardiovascular prognosis of nocturnal hypertensive patients.

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# TIME

# Best time for taking antihypertensive medication

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Night-time blood pressure shows a stronger association to future adverse cardiovascular outcome than values measured during the day, and there is an association between the early morning rise in blood pressure and cardiovascular events.[1,2] This has been taken to suggest that taking antihypertensive medication at bedtime may improve nocturnal blood pressure control and lower the risk for cardiovascular complication more than taking the medication in the morning. However, only few studies have been published in favour of this concept, and their results have been controversial.[3,4]

The recent publication of The Treatment in Morning versus Evening (TIME) study[5] provides important new information to this issue. This prospective decentralised randomised open parallel group study in the United Kingdom randomised patients 18 years or older, with diagnosis of hypertension, and treated by at least one antihypertensive medication to either take all medications in the morning or in the evening. All screening, consent, randomisation, and follow up was performed online or by email. Patients who had a home blood pressure monitor were asked to provide measurements at regular intervals. The primary outcome was the composite of vascular mortality and hospitalization for non-fatal myocardial infarction and stroke, and the events were adjudicated by a committee blinded for the randomisation group.

In all, 21 104 patients were randomised and 19 386 completed the study. Mean age was 65±1 years, 42% were female, 90% white, and last self-measured and reported blood pressure was135±13/79±9 mm Hg (as reported by 47% of the participants). The primary outcome during a median follow up was 5.2 years occurred in 3.4 and 3.7% in the evening and morning groups, respectively, with an unadjusted hazard ratio of 0.95 (95% confidence interval 0.83;1.10). Also secondary cardiovascular and mortality outcomes were similar in the two study groups. Adherence was reported throughout the study by 69%; and non-adherence at any time was greater in the evening group (39 vs 22%, P <0.0001). The evening group showed lower (1.8/0.4 mm Hg, P <0.0001) morning blood pressures and higher (1.1/0.9 mm Hg, P <0.0001) evening values, as compared to the morning group. While specific side effects were somewhat more common in the morning group, frequent visits to the toilet and non-specified adverse events were more common in the evening group. Reported falls was slightly less likely in the evening group with no difference in fractures between the two groups.

There are important limitations of this study.[5] This pragmatic decentralised study design was open, not closely monitored, data were self-reported and subject to bias, and information on blood pressure values were incomplete. Adherence was assessed by questionnaires, which is known to provide uncertain information. This notwithstanding, the TIME study provides important novel information. First, taking the medication in the evening did not reduce the risk for cardiovascular events or mortality, as compared to taking antihypertensive medication in the morning as is current practice in most cases. Second, the results of this large study do not support the unexpected findings published by Hermida et al[6], a study which must be interpreted with great caution.[4] Third, the pragmatic decentralised study design may, together with register-based randomised controlled studies[7], be one important way forward to use limited resources when conducting large randomised controlled studies.

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# TIME

## TIME for better science!

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Observational studies have repeatedly shown that night-time blood pressure better predicts future cardiovascular events than day-time blood pressure. Cardiovascular events more commonly occur during morning hours, and with many antihypertensive agents having suboptimal effect duration over the dosing period, it is well justified to investigate if blood pressure drugs should be taken at bedtime instead of in the morning.

In 2010, a Spanish group published the MAPEC study, showing a pronounced reduction in cardiovascular events in patients randomized to taking antihypertensive drugs at bedtime instead of taking the drugs in the morning.[1] This study in 2156 subjects did not receive much attention among researchers or general practitioners. In October 2019, the same research group published the Hygia Chronotherapy Trial in the European Heart Journal online.[2] The study included 19 084 subjects allocated to take their antihypertensive drugs in the morning or at bedtime. Taking antihypertensive drugs at bedtime was associated with pronounced reduction of cardiovascular events and total mortality compared with taking the drugs in the morning. Contrary to MAPEC, the Hygia study got extensive coverage in lay press with the third highest Altmetric score thus far in the journal. In the March 2020 issue of Hypertension News, we published a critical comment about how the study was reported, and presented a number of unsolved questions about the trial.[3] At the same time, several other researchers had similar criticisms, and in April 2020, the editors of European Heart Journal published an Expression of concern for the trial.[4]

Recently, the TIME (Treatment in Morning versus Evening) study with similar research question was published in *The Lancet* and is reported in this issue of Hypertension News.[5,6] The TIME study included 21104 patients randomized to take their antihypertensive drugs in the morning compared with bedtime and found no difference in the primary outcome of composite cardiovascular events between the two groups after a mean follow-up of 5.2 years. TIME had a pragmatic design, quite different from traditional efficacy trials. It is described as a "decentralized trial" without any in-person study visits. Screening, consent, randomization and follow-up, were done by the participants through a web site and e-mails. Subjects with a home blood pressure monitor sent in the results from their home blood pressure recordings (about half of the randomized subjects). The cardiovascular outcomes were recorded from the National Health Service data base.

We are thus in a situation where we have two large studies with similar research questions but very different results. How should one handle such a situation? Is it reasonable to pool the results from two trials with very different results in a meta-analysis to calculate the average effect? If not, how to choose which trial to trust?

The first step should always be to assess each trial based on its study type and the methodology reported. At first glance, both trials in the case of bedtime dosing are large randomized clinical outcome trials. There are numerous tools to assist critical appraisal of randomized controlled trials today, some directed towards clinicians, others, more formal, to be employed in systematic reviews and meta-analysis. Common features include details on the randomization procedure, potential sources of performance bias such as differential

follow-up between groups, and the assessment and reporting of clinical outcomes.

Whereas the TIME study describes, in detail, how informed consent was gathered through an online portal, after which the computer-randomized assignment was notified through e-mail, the randomization procedure is obscurely described for the Hygia trial, leaving questions about random sequence generation, as well as allocation concealment, unanswered. Furthermore, all primary and secondary outcomes are reported in raw numbers, as well as relative risks, in the main publication of TIME, whereas the actual number of events in Hygia is reported in an online comment to the main article, leaving the results section in the publication itself impossible to pierce. Thus, when scrutinizing the publications in more detail, it is obvious that the Hygia trial, with its poorly reported publication, enormous treatment effect, and many unanswered questions, is less likely to represent the true effect of bedtime dosing. On the other hand, TIME follows the general principles for how a clinical trial should be conducted and reported, and the results are therefore more likely to be reliable.

Usually, the more pragmatic a study is, the more likely the results are to reflect real life, usually with less prominent effect sizes than efficacy trials. This may of course be a limitation for trials with negative results – was the intervention indeed not effective or was the trial simply too pragmatic? The optimal study design to exclude an association between treatment and outcome is indeed a highly controlled efficacy trial. However, the results from TIME do in no way support the Hygia conclusions, and the TIME study must be regarded as the highest level of evidence so far.

From the above, it can easily be understood that pooling the results in a meta-analysis makes no sense. Figure 1 shows such an analysis, where the reader is advised to note the heterogeneity measure I-squared of 97%, indicating that the difference in results between the two studies is almost completely non-random. It is generally advised to be careful with meta-analysis if heterogeneity exceeds 50%, instead exploring potential differences between trials that may explain differences in results. In this particular case, we find different risk of bias between trials to be the most likely explanation.

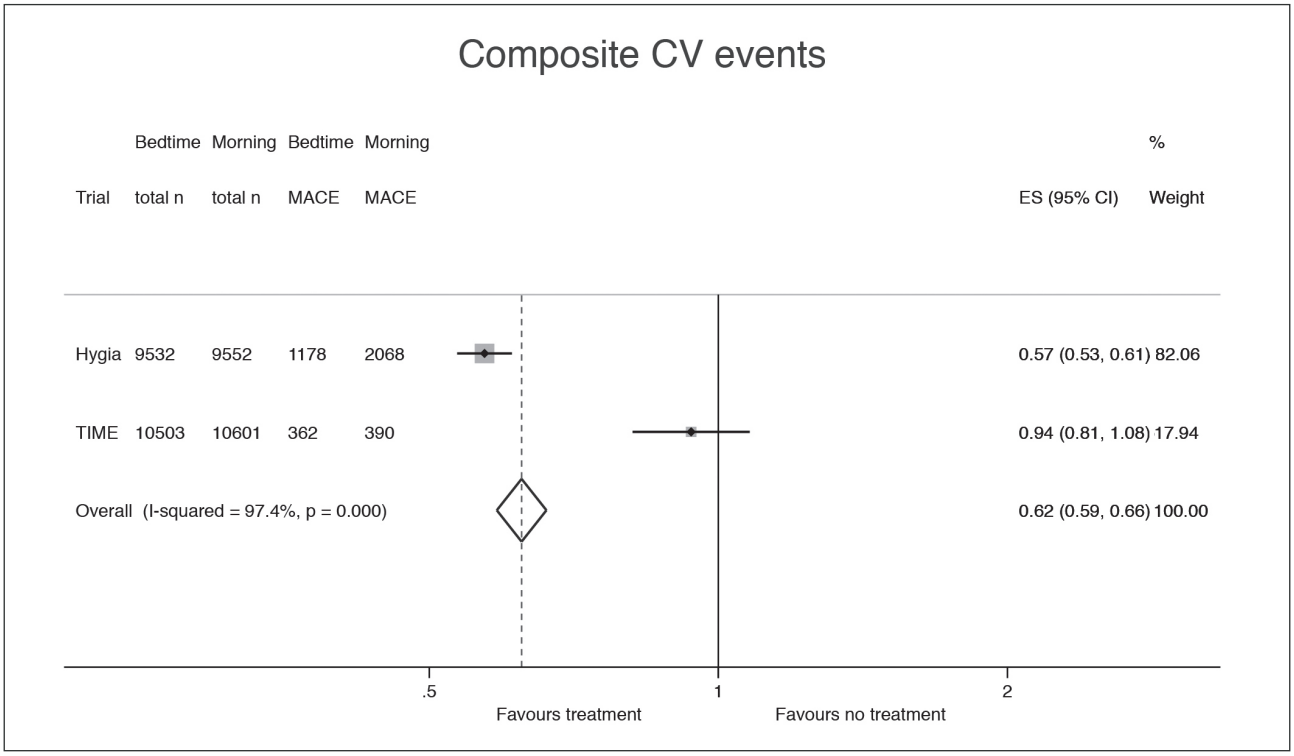


**Figure 1**. Meta-analysis of the primary composite cardiovascular outcome from Hygia and TIME. ES = effect size, here relative risk. CV = cardiovascular. MACE = major adverse cardiovascular event.

Fortunately, reporting problems like those found in Hygia are uncommon today, partly due to the wide application of reporting guidelines, such as the CONSORT guidelines for clinical trials published by the EQUATOR network. It is our impression that all major journals require EQUATOR checklists during the submission process. It is thus surprising that the high-ranked European Heart Journal, its editorial office, reviewers and editorial writers did not recognize the problems. Instead, in the printed issue, the article was chosen as "the Editors choice". On the other hand, during recent years, there have been a number of retractions of articles in most high ranked journals.

It must be stressed that the problems with the Hygia trial does not per se refute the bedtime dosing hypothesis but disqualifies inclusion of Hygia in the evidence base for bedtime dosing. More evidence about morning/bedtime dosing are in the pipeline. The Canadian BedMed Study plans to report their results in 2024.[7] Their BedMed-Frail study in elderly is also expected to be reported in 2024.[8]

What is to learn from the Hygia case? As readers and reviewers, our main issue is of course not to try to discover misconduct. However, it is important to try to understand the articles we read and not simply accept the reported findings at face value. If we do not understand the methods, most other readers will have the same problem and if the results are too good to be true, that may very well be the case.

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TIME

# ISH Position Papers 2020-2022 Bedtime dosing of antihypertensive medications

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**ISH Position Papers initiative**

ISH Position Papers is a new initiative introduced by Maciej Tomaszewski during his 2020-2022 ISH Presidency and led by Nadia Khan (Chair of ISH Research & Education Committee & Lead for Global Initiatives) and George Stergiou (Lead for ISH Position Papers). These are official ISH Statements on timely debatable clinical or basic science issues in hypertension and cardiovascular medicine. Authorship includes members of the ISH College of Experts, representatives by the ISH Regional Advisory Groups and other international experts. ISH Position Papers are endorsed by affiliated scientific organisations, including the World Hypertension League, the European Society of Hypertension, and potentially other organisations depending on the topic and aim for each paper.

The ISH Position Papers were first presented in a dedicated session during the 2022 ISH meeting in Kyoto on the 14th October (Table 1). They are usually published in the Journal of Hypertension (official ISH journal) accompanied by an animation video (https://www.youtube.com/watch?v=0z3tU_dj82M, https://www.youtube.com/watch?v=Memxc_gsdaU, https://www.youtube.com/watch?v=Yo98-ai3KhQ).[1-3]

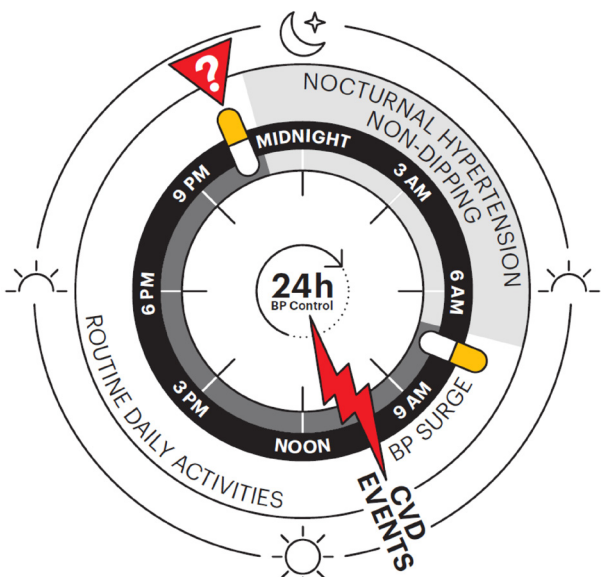
**Table 1:** ISH Position Papers 2020-2022 presented at the 2022 ISH meeting in Kyoto (14 Oct. 2022).

| ISH Position Paper | Lead & authors | Stage |
|---|---|---|
| 1. Virtual management of hypertension[1] | N. Khan + 16 authors | Published Mar. 2022 |
| 2. Bedtime dosing of antihypertensive medications[2] | G. Stergiou + 23 authors | Published Aug. 2022 |
| 3. Lifestyle management of hypertension and cardiovascular disease | F. Charchar + >30 authors | On preparation End 2022 |
| 4. Combination therapy for hypertension | B. Williams + >20 authors | On preparation 2023 |
| 5. Addressing global disparities in blood pressure control[3] | A. Schutte + 38 authors | Published Oct. 2022 |

## Bedtime dosing of antihypertensive medications: Theoretical background

In 1998, Eoin O'Brien introduced the terms "dippers" and "non-dippers" to describe hypertensive individuals with considerable decline in blood pressure (BP) during night-time sleep as compared to the daytime levels.[4] At that time, he was able to show than non-dippers have higher risk of stroke and called for further research to determine the prognostic and therapeutic implications of his observations.[4] Regrettably, longer than three decades later, his questions have not been addressed with adequate research data. In 1999, in an analysis of the Syst-Eur study Jan Staessen showed that the night/day BP ratio assessed by 24h ambulatory monitoring provided additional prognostic information beyond that of the 24h average.[5] Last, in 2005 Giuseppe Mancia showed than in the PAMELA study night-time ambulatory BP was a stronger predictor of death than daytime ambulatory, home, or office BP.[6] These findings were confirmed by other studies.[7] Thus, a challenging hypothesis has been generated. As night-time BP and non-dippers are so important, and there is a surge in BP and cardiovascular events in the morning hours[8], why not administer the antihypertensive medications at bedtime aiming to reduce the night-time BP, prevent the morning BP surge and reduce cardiovascular events (Figure 1).

**Figure 1.** Theoretical background favouring bedtime dosing of antihypertensive medications.




## Bedtime dosing of antihypertensive medications: Outcome data

The 2022 ISH Position Paper on "Bedtime dosing of antihypertensive medications"[2] presents a systematic review of 713 potentially relevant PubMed publications of which 8 were eligible for analysis, including more than 55,000 participants with 2-6 years follow-up.[2] Evaluation of their design and methodology showed that although some of them were adequately designed for addressing each one of their primary endpoints, all of them had a high risk of bias for assessing the impact of bedtime dosing on outcome. Thus, 5 studies were deemed "inadequate" and 3 "partially adequate" to inform on the "bedtime dosing" research question. Two of the 3 partially adequate studies, which were organised by a single research group in Spain, were the only ones strongly favouring bedtime dosing. However, these studies received a lot of criticism for methodological integrity and transparency (see sister paper by Bo Carlberg and Mattias Brunström in this issue of ISH News).[9,10] Unfortunately, despite the very questionably data supporting bedtime dosing, the Spanish data have been widely distributed and influenced healthcare professionals and the public.

The TIME study in UK and the BedMed and BedMed-Frail in Canada are outcome studies designed to address this research question. The results of the TIME study were recently published[11] (see sister paper by Thomas Kahan in this issue of ISH News). In brief, the TIME study included 21,134 treated hypertensives followed for 5,2 years and showed no benefit (or harm) by taking all antihypertensive medications in the evening versus in the morning.[11] These findings fully support the 2022 ISH Position Paper recommendations.[2]

**2022 ISH Position Paper on "Bedtime dosing of antihypertensive medications": Key recommendations[2]**

• A key aim of BP lowering treatment should be to achieve complete 24-h control of BP by using long-acting antihypertensive medications.

• Bedtime drug dosing should not be routinely recommended in clinical practice.

• Treatment of hypertension should be based on long-acting agents in monotherapy or in combinations administered in a single morning dose, as applied in the vast majority of long-term outcome hypertension trials.

• Whether for selected patients with confirmed nocturnal hypertension and concomitant disease (e.g., cardiovascular disease, chronic kidney disease, sleep apnea, or diabetes mellitus), there might be a benefit from tailoring treatment to control nocturnal BP, needs further investigation.

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# LEARNING THE ROPES FROM BENCH TO CLINIC: NITRATES IN VASCULAR BIOLOGY

## Introduction

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Nitrovasodilators have been used in the treatment of cardiac conditions such as angina, infarction and heart failure ever since Thomas Lauder-Brunton had introduced amyl nitrate into the therapeutic armamentarium in 1867.[1] However, he was by no means the first to make therapeutic use of nitrates: In a text dating back to the fifth to sixth century, the Chinese alchemist and physician, Tao Hongjing, reported that salpeter (KNO3) powder placed under the tongue would alleviate chest pain and other conditions of cardiovascular distress.[2] Today, we know that bacteria present in the saliva would convert salpetre into nitrite which would then give rise to NO or may act via other pathways.

More than one-hundred years after Lauder-Brunton, with the discovery and functional description of different vasodilating factors released from the vascular endothelium[3], research into these compounds has gained an immense momentum. I still remember the incredulous astonishment within the scientific community when it became clear that the so-called endothelium-derived relaxation factor (EDRF) was nothing more than nitric oxide (NO).[4] That the endothelium would release a dissolved gas towards the adjacent vascular smooth muscle layer to induce vascular relaxation was difficult to reconcile with current concepts of the time. Robert Furchgott shared the Nobel Price for the discovery of this new cellular signal with Louis Ignarro and Ferid Murad in 1998. Salvatore Moncada, whose group had independently also identified EDRF as NO, was not awarded. With the work of these researchers and their groups, the question as to the long-sought mechanism of vasodilation by nitrates including nitroglycerin had been answered.

Today, compounds stimulating the intracellular NO vasodilating system, modifying, among others, the generation and activation of the NO-binding soluble guanylyl cyclase (sGC), are subject of intensive drug research and have yielded, among many others, the drug sildenafil, commonly known as Viagra.

A multitude of agents have the capacity to induce NO in endothelial cells. For instance, when working on the functional role of angiotensin receptors, we discovered that the angiotensin AT2 receptor, the intrinsic antagonist of the vasoconstrictor angiotensin AT1 receptor, induces vasodilatation and other effects by intracellular NO generation via a kinin-dependent pathway.[3] However, the vascular endothelium is not only capable of releasing vasodilators but can also do the opposite: generate and release vasoconstrictors such as endothelin.[6,7] It appears that the balance or disbalance between vasodilating and vasoconstricting endothelial factors contributes essentially to vascular physiology or pathophysiology such as in hypertension.[8]

Research has moved on since NO was described as the EDRF and has given us a plethora of new findings. In the current section of "Learning the Ropes", four authors, who have significantly contributed to NO/nitrate/nitrite research, contribute state- of-the-art articles on results and their thoughts in this intriguing field.

**Miriam Cortese-Krott** opens up with some fundamental aspects of the rather complicated NO/nitrate/nitrite story focusing on basic mechanisms of endogenous NO generation. She explains how

nitrite and nitrate have changed in the view of researchers from low-interest byproducts to important precursors of the vasodilator NO. Among other nutrients, we learn about (root) beets as a promising exogenous source of nitrates which we will encounter again in the following articles. Striking new findings on the role of red blood cells (RBCs) and hemoglobin-α in producing NO under hypoxic conditions are also presented.

This is followed by **Thomas Münzel** and **Andreas Daiber** detailing on the beneficial effects of organic nitrates in cardiac conditions like coronary ischemia and heart failure. They also report on the unwarranted phenomenon of nitrate tolerance and explain why nitrates have no place in the long-term treatment of arterial hypertension but are useful in uncontrolled hypertension and hypertensive emergencies. In addition, they familiarize us with the relevant recommendations in the guidelines of the ESC, a major international cardiological society.

**Mattthias Carlström** and **Josefine Nasiel** report on dietary nitrate supplementation in hypertension-associated with pregnancy, a topic which is not always on the screen of practicing physicians. They touch upon the role of the NO synthase system (NOS) in preeclampsia, and on the importance of oxidative stress in endothelial dysfunction with reduced NO production. Further, they demonstrate that in experimental models of hypertension in pregnancy, dietary supplementation of nitrate with beetroot juice or nitrite could prevent adverse outcomes and that these experimental findings were confirmed in part in pregnant women raising hopes that chronic dietary nitrate supplementation might become a therapeutic tool in preeclampsia.

Finally, **Vikas Kapil** and **Amrita Ahluwalia** educate us on the knowns and unknowns in the realm of nitrates/nitrites and NO. Besides "canonical", there exist also non-canonical" pathways of NO generation. For instance, nitrite may not always act through the generation of NO but possibly also through nitrosothiols and nitrated fatty acids, and even by itself. They close by acknowledging that "nitrate supplementation leads to authentic NO production and blood pressure reduction" but confess that "true clinical translation of these knowns is currently absent."

While this statement is certainly true as well as the conclusion by Münzel and Daiber that it remains so far unclear how nitrate therapy changes prognosis of our patients, we should not be deterred from acknowledging the enormous progress that has been made in the field, and also that nitrate therapy is still very useful in several cardiac conditions, especially angina. Results with anorganic nitrites in hypertension and preeclampsia are also promising and will certainly stimulate more extensive clinical research.

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# LEARNING THE ROPES

## NO, nitrites and nitrates and their role in vascular control - New fundamental developments in basic and translational research

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**Nitric oxide and regulation of vascular relaxation**

Compared to other transmitters in the body, NO has very peculiar characteristics. It is an uncharged, small "free" radical and a dissolved gas (i.e., NO is a solute in biological liquids and not a gas). NO has a lower reactivity and a longer half-life in solution as compared to other charged and more reactive "free radicals" like superoxide radical anion ($O2^{\bullet-}$) and other oxidants like H2O2. Being uncharged and small, NO can easily cross membranes without needing transporters or channels, and work as a transmitter for cell-cell communication

In the vasculature, nitric oxide (NO) is produced in endothelial cells by the endothelial isoform of nitric oxide synthase (eNOS) and regulates vascular tone[1] (Fig. 1). NO-mediated activation of the sGC/PKG pathway also inhibits platelet activation. In this way, endothelial NO contributes to maintaining the vascular tone and hemostasis. NO can also undergo oxidation reactions forming nitrite and nitrate[2] (Fig. 1) Nitrate and nitrite are anions and do not readily cross cell membranes without the help of transporters or channels.

**Nitrite and nitrate: from inert products of NO degradation to precursors of NO production**

Initially nitrite and nitrate were considered by medical scientists as uninteresting byproducts of NO oxidation showing very low reactivity and vasoactivity. High nitrate concentrations were though to induce the production of nitrosamines and considered cancerogenic. Views have radically changed since then.[1,3] Nitrite and nitrate are now seen as precursors of NO in a synthetic pathway which is independent from eNOS activity. The nitrate-nitrite-NO pathway was shown to participate in regulating vascular tone, modulating blood pressure, and protecting from ischemia-reperfusion injury of the heart, liver, and kidney.[1]

The sources of nitrates in the body can be endogenous (NO oxidation) or exogenous (i.e., food like lettuce, spinach, rocket/arugula, beets and radish, and cured meat) while body nitrite is endogenously produced and is used as a food preservative. Nitrate can be actively transported and concentrated into the saliva by ion pumps.[4] In the mouth, it can be converted back into nitrite and NO by bacterial nitrate reductase and then adsorbed into the circulation or swallowed to reach the stomach and the gut. Interestingly, antibacterial mouthwash inhibits the blood pressure-lowering effects of oral nitrate.[5] In the gut, bacterial nitrate/nitrite reductases in the microflora can further convert nitrate into nitrite and NO. In the acidic environment of the stomach, nitrite ($NO_2$-) and nitrate ($NO_3$-) can be protonated to the unstable nitrous ($HNO_2$) and nitric ($HNO_3$) acids that may decompose into nitrogen species (like $N_2O_3$ and $NO_2$) and NO. These may lead to nitrosation reactions (formation of nitrosothiols and nitrosamine) and nitration. Recently, it was shown that administration of the proton pump inhibitor esomeprazole inhibited the antihypertensive effects of oral nitrite but not the effects of NO donors or vasorelaxants in humans.[6]

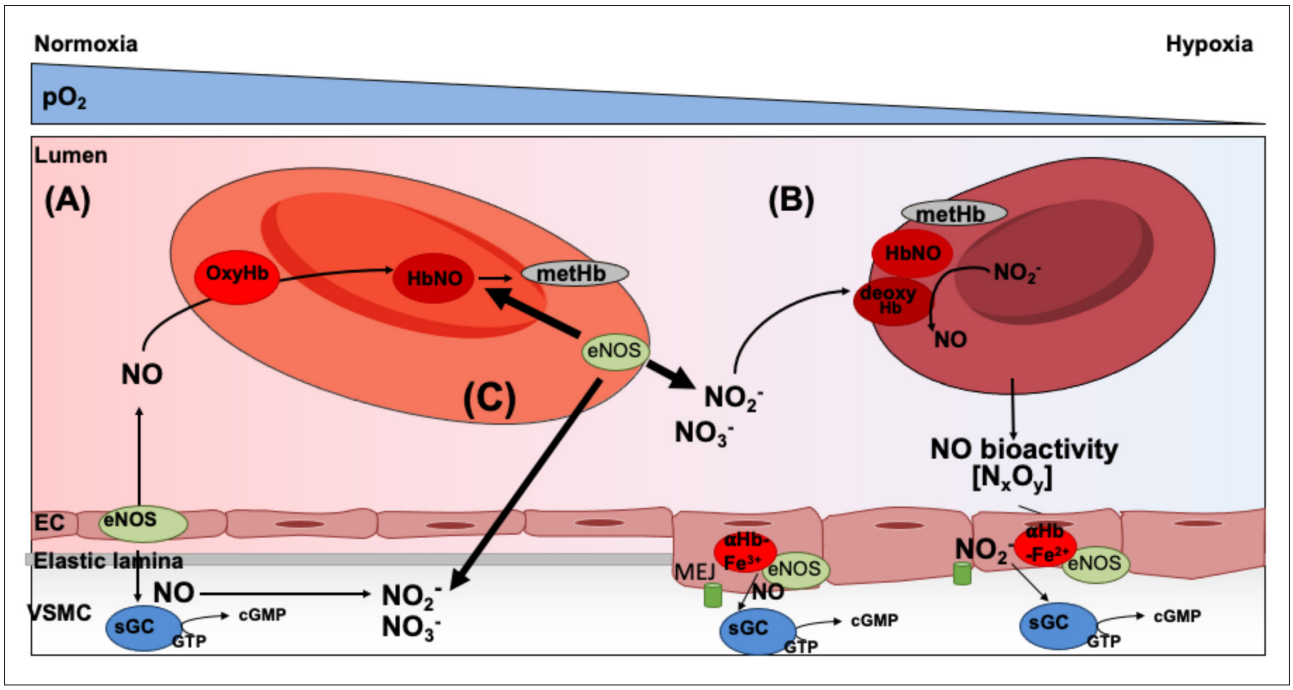


**Figure 1**: Nitric oxide, nitrite, and nitrate and their role in vascular control. The endogenous source of circulating nitrite ($NO_2^-$) and nitrate ($NO_3^-$) under healthy conditions is the endothelial nitric oxide synthase (eNOS) expressed in endothelial cells (ECs). In the vessel wall, NO activates the soluble guanylate cyclase (sGC), which is expressed in smooth muscle cells (VSMC) and induces the production of cGMP from GTP. NO can be oxidized to nitrite and nitrate in enzyme-catalyzed reactions in the tissue and the plasma. In the red blood cells under normoxic conditions, NO reacts with oxyhemoglobin (oxyHb, $Fe^{3+}$-Hb) to produce nitrate and methemoglobin (metHb, $Fe^{3+}$-Hb). Red blood cells express an active eNOS, which contributes to the levels of HbNO and circulating nitrite and nitrite levels in plasma. (B) In resistive vessels and arterioles, the endothelial cells are directly connected to smooth muscle cells by a myoendothelial junction (MEJ), which expresses eNOS and hemoglobin-α (Hb-α). The two protein form a functional complex regulating the NO transfer into the smooth muscle cell. NO can pass through only if Hb-α is oxidized (αHb- $Fe^{3+}$). Instead, while in its reduced form αHb-$Fe^{2+}$ scavenges NO and produces nitrate. At low $pO_2$, hemoglobin red blood cells were shown to induce hypoxic vasodilation. Under these conditions, red blood cells produce NO from nitrite in a reaction catalyzed by deoxyhemoglobin (deoxyHb, $Fe^{2+}$-Hb). The same reaction occurs in the MEJ where nitrite is converted onto NO by αHb-$Fe^{2+}$, thus contributing to hypoxia-induced vascular control.

In blood, red blood cells (RBCs) convert nitrite into NO under hypoxic conditions leading to vasodilation.[7] In tissues, nitrite was shown to be converted into NO under hypoxic conditions in a reaction catalyzed by metalloenzymes like globins (hemoglobin, myoglobin, neuroglobin, cytoglobin)[3,4], other heme proteins (e.g., eNOS, cystathionine β-synthase), and by molybdenum–molybdopterin containing enzymes (xanthine oxidase, aldehyde oxidase, sulfite oxidase and mitochondrial amidoxime-reducing component–1 and 2).[3] Beside the bacterial nitrate reductase, nitrate is metabolized by mammalian xanthine oxidoreductase and therefore is – like nitrite - an endogenous precursor of NO formation, at least in the liver.[8]

The skin microflora can convert nitrate into nitrite. Even sunlight was shown to convert nitrite into nitrosospecies and NO, which correlates with changes in circulating NO metabolites in plasma and a decrease in blood pressure in humans.[9,10] Lack of sunlight may increase the risk of hypertension and cardiovascular disease by impairing NO production in the skin.[9]

## Role of hemoglobin-α in control of NO bioactivity in resistance arteries

In resistance arteries and arterioles, the endothelial cells are connected with smooth muscle cells through protrusions defined as myoendothelial junctions (Fig. 1), which allow direct cell-cell communication.[11] Notably, the release of NO from the endothelium to the smooth muscle cells depends on the redox state of α-hemoglobin: It will scavenge NO, when in its reduced ($Fe^{2+}$), NO binding form, or let it pass through the myoendothelial junction, when it is in its oxidized ($Fe^{3+}$) form, as the oxidized ($Fe^{3+}$) form cannot bind NO

(Fig. 1). However, like all globins, the α-hemoglobin chain can also convert nitrite into NO under hypoxic conditions.[4] Indeed, endothelial-specific α-hemoglobin knock-out mice showed significantly decreased exercise capacity, decreased hypoxia-induced vasodilation, and lacked the hypotonic effects induced by hypoxia, compared to wild-type mice.[12] Therefore, α-hemoglobin may provide local NO from nitrite in response to hypoxia, thus allowing to match oxygen delivery to demand in tissues like the skeletal muscle.

**Role of red blood cells in control of systemic NO metabolism and vascular tone**

For a long time, RBCs were thought to limit the NO bioactivity in the blood and its bioavailability for vasodilation (Fig, 1). Instead, RBCs are major regulators of NO bioavailability. On one side, normoxic NO scavenging contributes to fine-tuning hemostasis and vasodilation; on the other side, under hypoxic conditions, RBCs induce vasodilation and convert nitrite into NO.[2] Thus, hypoxia-induced release of NO bioactivity is necessary for matching oxygen delivery to demand.[2]

RBCs express also a complete eNOS/sGC/PKG pathway.[13] We recently generated loss-of-function and gain-of-function mice models to specifically knock out and knock-in eNOS in the RBCs.[14] Surprisingly, mice lacking eNOS only in RBC showed decreased circulating nitrite and nitrate in the plasma, increased blood pressure, and systemic vascular resistance but fully preserved eNOS-dependent vascular endothelial function. Vice versa, when eNOS expression was restored in RBCs only, the presence of eNOS in RBCs rescued global eNOS knock-out mice from hypertension.[14] These data demonstrate that RBC eNOS plays an unexpected role in regulating nitrite and nitrate levels, systemic vascular resistance, and blood pressure.

**Summary and outlook**

Nitrite and nitrate are sources for endogenous NO and may act as signaling molecules on their own. The role of RBCs in regulating systemic NO metabolism and blood pressure independently from vascular eNOS is very intriguing and may open new perspectives for understanding, preventing or treating hypertension.

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# LEARNING THE ROPES:

## Organic nitrates: Indications for the treatment of patients with hypertension and cardiac disease, and tolerance development

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Organic nitrates still remain useful drugs in the treatment of acute and chronic ischemia and heart failure alone or in combination with arterial hypertension but also in the prevention of exercise-induced angina, although almost all studies failed to demonstrate any prognostic benefit in response to long-term therapy. Important pathomechanisms being responsible for this phenomenon may include side effects like nitrate tolerance or nitrate induced endothelial dysfunction as well as oxidative stress.

### Organic nitrates hemodynamic actions

Nitrates act mainly on venous capacitance vessels, large- and medium-sized coronary arteries, collaterals (Figure 1), and also the aorta, while coronary and peripheral arterioles with a diameter <100 µm have been demonstrated to be nitrate resistant.[1] Thus, e.g. in patients with chronic coronary syndrome, the powerful dilation of veins by nitrates (venous pooling) causes a preload reduction, which will reduce end-diastolic filling pressure of the left ventricle as well as wall tension and thus myocardial workload and oxygen demand.

### Cellular mechanisms of vasodilation

Organic nitrates release nitric oxide (•NO) or a related compound in response to intracellular bioactivation (for glyceryl trinatrate [GTN], the mitochondrial aldehyde dehydrogenase [ALDH-2][1,3] and activation of the target enzyme, the soluble guanylyl cyclase (sGC).[3,5] Increased concentrations of cGMP in turn will cause the activation of the cGMP-dependent kinase I, which primarily induces a decrease in intracellular calcium in smooth muscle cells and subsequently the relaxation of the vascular smooth muscle by diminished contractility of the myosin-actin filaments.

### Organic nitrates in arterial hypertension

According to the global burden of diseases, arterial hypertension ranks among the cardiovascular risk factors number one as a risk factor for death.[2] The use of organic nitrate to restore NO to the vasculature in hypertensive individuals or hypertensive rodents began about a century ago. Their main antihypertensive effect is a combination of venodilation and dilation of large conductance vessels such as the aorta. By reducing pulse wave

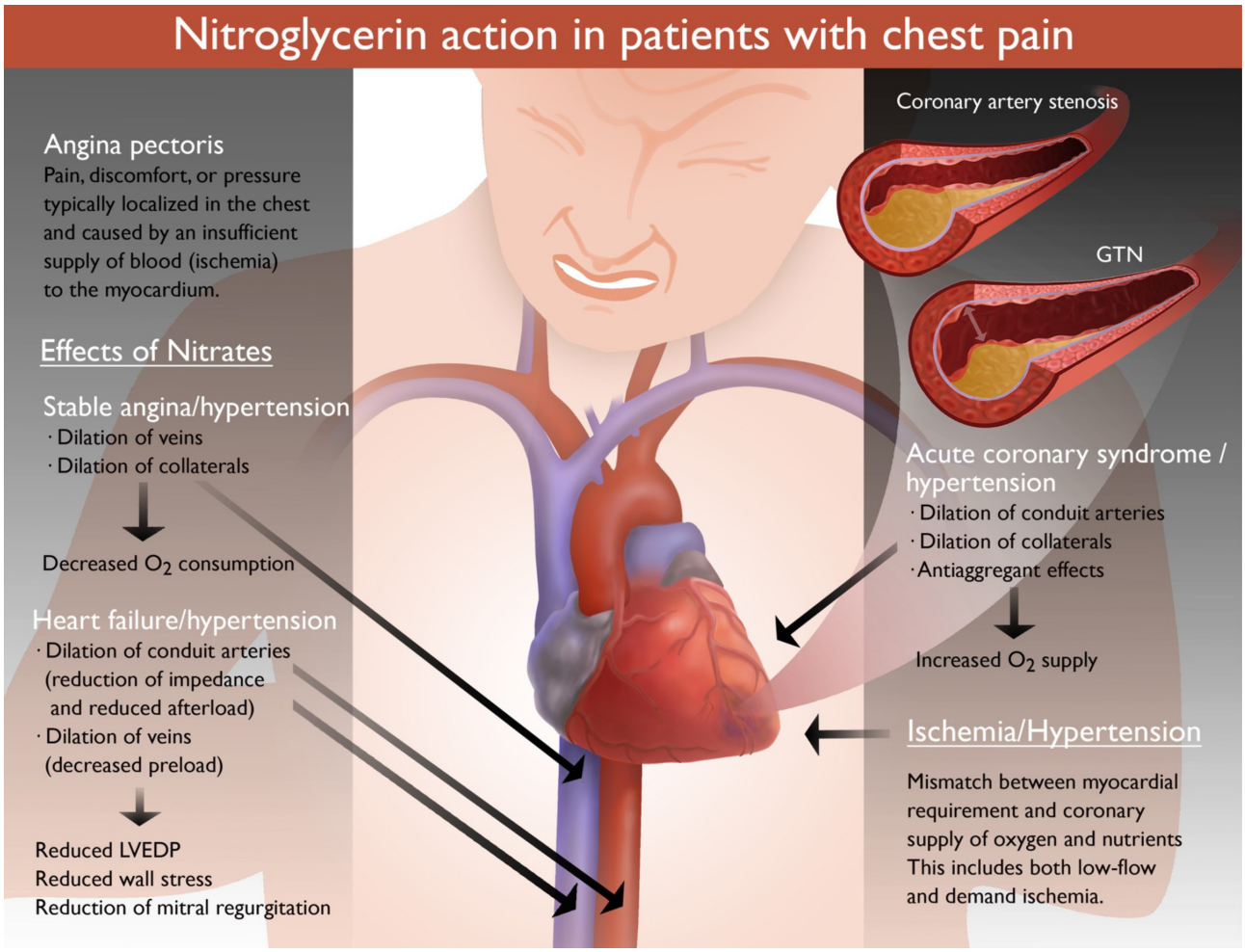


**Figure 1:** Hemodynamic effects of acutely administered nitroglycerin (GTN). Reused from[1] with permission.

velocity this will reduce mainly systolic blood pressure. However, due to the rapid development of tolerance and the acuteness of the vasorelaxant response[3], the main problems also associated with organic nitrates, the use of nitrates today is limited to the treatment of the hypertensive crisis, while their long-term use for blood pressure reduction is not considered a feasible first-line option. Consequently, in the 2020 global hypertension guidelines of the ISH[4], nitrates are not mentioned for chronic oral treatment of hypertension. An i.v. solution GTN or sodium nitroprusside can be given to treat patients with hypertensive crisis or patients with hypertension and pulmonary edema.

### Anti-ischemic actions of organic nitrates

In patients with acute coronary syndrome (ACS), organic nitrates have potent anti-ischemic effects that are caused by the dilation of large epicardial coronary arteries (in particular in areas with eccentric stenosis) and coronary collaterals.[1] The consequence is improved blood perfusion and thus improved delivery of oxygen to subendocardium. Because arteriolar tone is largely unaffected by organic nitrates, one rarely encounters reflex tachycardia or even coronary steal phenomena. Thus, the short-term nitrate administration is able to correct acutely the mismatch between oxygen demand and oxygen supply in ischemic cardiac tissue in subjects with angina pectoris.

### Benficial hemodynamic effects of organic nitrates in patients with heart failure

In patients with acute and chronic congestive heart failure (CHF), organic nitrates will also markedly improve left ventricular function.[1] Because of the preferential venodilation, nitrates will reduce the right atrial pressure followed by a redistribution of blood volume from the central circulation into larger capacitance veins. Nitrates will also reduce the impedance (phasic resistance) to the afterload dependent left ventricle and thus will

cause an unloading of the ventricle via dilation of large capacitance arteries (aorta). The increase in compliance of the arterial vasculature in turn leads to a reduction in the magnitude, frequency, and velocity of reflected waves to the left ventricle. These beneficial effects on afterload induced by nitrate therapy will cause an increase stroke volume, a reduction of the filling pressure and wall tension of the left ventricle, and if present, also a reduction in the degree of mitral regurgitation.

**Clinical use of organic nitrates and their recommendation of use in the ESC/ESH guidelines**

The ESC/ESH guidelines for the treatment of hypertension recommend GTN i.v. as a first line therapy in patients with hypertensive emergencies and ischemic heart disease and aortic dissection in both situations in combination with beta-blocker therapy.[6] These guidelines further suggest the use of long-acting nitrate (LAN) as a second step treatment for patients with chronic coronary syndrome.[7] For patients with acute coronary syndromes there are IC indications for the use of sublingual or i.v. nitrates and early initiation of beta-blocker treatment in patients with ongoing ischemic symptoms and without contraindications.[8]

Organic nitrates are further recommended in patients with uncontrolled hypertension or signs of heart failure (IC).[9] In patients with suspected/confirmed vasospastic angina, calcium channel blockers and nitrates should be considered (IIA).[7]

In CHF patients, a combination therapy of hydralazine and isosorbide dinitrate (ISDN) may be given (IIA) plus conventional therapy or in patients with HFrEF being intolerant to therapy with an angiotensin-converting enzyme (ACE)-inhibitor, AT1-receptor blocker or angiotensin receptor II blocker - neprilysin inhibitor (ARNI).[9] The positive study in subjects with heart failure (A-Heft Trial), however, demonstrated survival benefits just for African Americans.[10]

Despite the beneficial acute hemodynamic effects of nitrate therapy, the prolonged treatment of patients with acute heart failure with nitrates either given as a bolus or i.v. treatment, did not yield a survival benefit compared with e.g. high dose diuretic therapy.[11]

It is also interesting to note that data from the GRACE registry suggest that therapy with nitrates may cause a shift from ST-elevation to non-ST elevation myocardial infarction,[12] implicating a positive impact of nitrates on the pathophysiology of plaque rupture and/or a preconditioning-like effect.[1,3]

**Complications of nitrate therapy: the phenomenon of nitrate tolerance and endothelial dysfunction:**

The chronic administration of organic nitrates will trigger counter-regulatory vasoconstrictor mechanisms in response to vasodilation that are characterized by increased levels of vasoconstrictors such angiotensin II, endothelin-1 and noradrenalin and increased oxidative stress and endothelial dysfunction.[1,3] This indicates that beyond their pharmacological interest at the molecular level, these nitrate-induced side effects might also have important implications in patients where endothelial dysfunction and oxidative stress is already encountered such in patients with CAD, hypertension, and heart failure.[5]

Every effort at identifying a single mechanism responsible for nitrate tolerance has failed in the last 40-50 years: the mechanisms are likely to be multifactorial and may include at the systemic level [neurohormonal activation and intravascular volume expansion, so-called pseudo-tolerance] as well as more specific vascular disturbances (so-called true vascular tolerance), such as the inhibition of nitrate biotransformation by the ALDH-2 (operative for GTN and pentaerithrityl tetranitrate [PETN]), desensitization of the soluble guanylyl cyclase, increase in phosphodiesterase activity, and uncoupling of the NO synthase leading to cross tolerance to other NO-donor substances and classical endothelial dysfunction.[3] These side effects concern GTN, ISDN and isosorbide-5-mononitrate (ISMN) but not PETN, which has interestingly powerful stimulatory effects on antioxidant enzymes such as the heme oxygenase-1.[1,3] Mechanisms that have been suggested to prevent tolerance and nitrate-induced endothelial dysfunction in response to GTN, ISDN and ISMN may include a nitrate free interval or concomitant therapy with hydralazine, ACE-inhibitors, AT-1 receptor blockers or statins.

## Summary

Patients should be educated on the use of short-term sublingual formulations in the situational prophylaxis of angina: self-administration of GTN or ISDN before physical or emotional stress may indeed improve both exercise tolerance and quality of life. Finally, more research is necessary on alternative NO-based therapies, for instance the administration of nitrite/nitrate[13] or PETN.[5] While basic science has provided more answers to the still unresolved question of nitrate tolerance[3], the most important question, i.e. how does this therapy change patient prognosis, remains so far negative.

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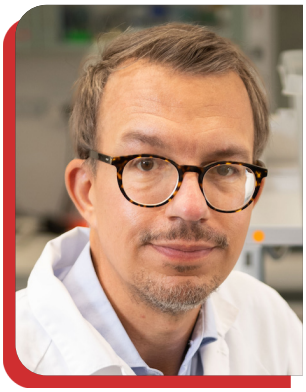
# LEARNING THE ROPES

## Therapeutic value of dietary nitrate supplementation in pregnancy-related hypertensive disorders



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### Hypertensive pregnancy disorders

Cardiovascular disease (CVD) including hypertension accounts for significant morbidity and mortality. Despite significant research and clinical efforts during the last decades, the global prevalence of CVD has increased and is estimated to further increase.[1] These trends are mirrored in pregnant women globally, which increases the risk of adverse cardiovascular complications of pregnancy, such as preeclampsia (PE), which in turn, predisposes both the mother and offspring to increased future cardiovascular risk.[2] This undesirable epidemiological shift in the occurrence of CVD and hypertension in the modern society can be largely linked to a lifestyle with increased sedentarism and an unhealthy diet. This poses a great challenge for the healthcare system, and identification of novel therapeutic targets for the prevention of future cardiovascular disorders are urgently required. This is particularly important in high-risk populations, such as pregnant women with gestational hypertension and PE, where treatment options are limited. Hypertensive disorders during pregnancy are serious cardiovascular problems that affect more than 10% of all pregnant women globally, where the PE syndrome is the leading cause of perinatal and maternal morbidity and mortality. Currently, the only treatment option in severe cases of PE is delivery and many times preterm delivery. This is the leading factor causing neonatal mortality and morbidity resulting in not only adverse health outcomes for mother and baby, but also significant healthcare costs.

The PE syndrome is characterized by new-onset hypertension and abnormal function in one or several organ systems post 20 weeks of gestation. The pathophysiology coupled to the development and progression of PE are complex, involving different stages with defective trophoblast invasion and reduced ueteroplacental perfusion, which in turn can trigger a stressed placenta. Suggested underlying pathogenic mechanisms involve increased oxidative stress coupled with reduced production and/or signaling of nitric oxide (NO) which contributes to the development of endothelial dysfunction and further blood pressure elevation.

### Nitric oxide synthase system

The small and gaseous molecule NO is vital for several functions in the cardiovascular system, including modulation of angiogenesis, platelet aggregation, endothelium-derived relaxation, and blood pressure control. During normal pregnancy, NO is crucially involved in the adaptation to the systemic increases in blood volume, cardiac output and decreased vascular resistance. Classically, NO is generated enzymatically from the NO synthase (NOS) system where endothelial NOS (eNOS) is of outmost importance in the vasculature. During

aging and several lifestyle-associated disorders including CVD, NO bioavailability is reduced. Disruption of the delicate balance between NO and reactive oxygen species (ROS), including superoxide, leads to a vicious pathogenic cycle which results in oxidative stress and endothelial dysfunction, key features of numerous cardiovascular disorders including hypertension. In PE, although there have been conflicting reports, abnormal maternal NO homeostasis has been demonstrated clinically as evidenced by reduced NO bioavailability in the circulation[3] coupled with reduced flow-mediated dilatation (FMD)[4] compared with healthy pregnancies.

**Oxidative stress**

Oxidative stress is considered a central pathogenic component that contributes to the development and progression of endothelial dysfunction in PE. The ischemic or hypoxic environment of the utero-placental circulation induced by placental dysfunction, in combination with pro-inflammatory processes, is reported to be an early stimulus of excessive ROS generation. Indeed, studies have reported multiple placental sources of ROS, including the mitochondria, the NADPH oxidase, and the xanthine oxidase systems as well uncoupled eNOS that produce superoxide rather than NO. Clinical studies using dietary vitamin C and E supplementation have in general failed to show any favorable effects in CVD and in PE.[5] The explanation for this is not yet clear, but is likely associated with the relatively poor systemic antioxidant effect when they are administered orally and simply do not reach the cellular target which is the origin for the oxidative stress and the reduced NO availability. It is speculated that novel treatment strategies that efficiently dampen oxidative stress and restore NO production or its downstream signalling may have therapeutic value by preventing the development and progression of hypertensive pregnancy disorders including PE.

**Nitrate-nitrite-nitric oxide pathway**

In addition to the enzymatic eNOS pathway, a fundamentally different system for NO generation exists, whereby inorganic nitrate ($NO_3$-) is serially reduced to nitrite ($NO_2$-) and NO in blood and tissues. This system, referred to as the nitrate-nitrite-NO pathway, may be boosted via our daily diet. High levels of nitrate are found in many vegetables, including leafy greens and beets, i.e., food groups associated with reduced cardiovascular risk. Earlier studies studies in healthy adults and in patients with hypertension have shown that dietary nitrate supplementation can lower blood pressure and improve endothelial function, at least in part via reduction of oxidative stress and increasing NO bioavailability.

Importantly, reduction of nitrite to NO via the NOS-independent pathway is enhanced under hypoxic conditions, therefore, has the potential to alter NO bioavailability in the hypoxic utero-placental circulation during PE. Thus, dietary supplementation with inorganic nitrate or nitrite may provide beneficial effects for both the mother and fetus. One such approach is consumption of concentrated beetroot juice that contains high levels of nitrate.

**Dietary nitrate interventions**

In vivo experimental studies using genetically eNOS deficient mice or mice with pharmacological inhibition of NOS have demonstrated that a dysfunctional eNOS system importantly contributes to the development of a PE-like phenotype during pregnancy, e.g., hypertension, endothelial dysfunction, and intrauterine growth restriction (IUGF). Dietary supplementation with beetroot juice or nitrite was shown to lower blood pressure, preserve antioxidant function and prevent adverse pregnancy outcomes in these two hypertension-in-pregnancy models.[6,7]

In a randomized clinical trial in pregnant women, a single dose of beetroot juice improved macrovascular endothelial function (FMD), but did not significantly change tissue oxygenation.[8] In a clinical feasibility study, safety and acceptability of dietary nitrate supplementation via 70 mL concentrated beetroot juice (equal to 400 mg nitrate) in borderline hypertensive pregnant women (>22 weeks gestation) was demonstrated.[9] Although no significant difference in blood pressure was demonstrated between nitrate and placebo groups, this was attributed to the lack of dietary control and in turn, increased plasma nitrate and nitrite. Indeed, changes in plasma nitrite levels were linearly correlated to decreases in diastolic blood pressure.[9] Moreover, the same group used isolated and pre-constricted chorionic plate vessels from women with normal and intrauterine growth

restriction (IUGR) pregnancies, and demonstrated that enhanced nitrite-mediated relaxation of placental blood vessels exposed to hypoxia was preserved in pregnancies complicated by IUGR.[10] Taken together, this suggests that interventions targeting the nitrate-nitrite-NO pathway have the potential to improve fetoplacental blood flow in IUGR pregnancies.

**Conclusion and future perspective**

The lack of effective therapeutic approaches for PE, concomitant with the evidenced oxidative stress and dysfunctional NO homeostasis as well as hypoxic utero-placental circulation, provides considerable scope for the evaluation of chronic dietary nitrate supplementation. This nutritional approach may contribute to improved endothelial function, reduction of blood pressure and improvement of gestational outcomes for the mother and the child, that may reduce both the mortality, as well as acute and chronic cardiovascular morbidity, associated with PE (Figure 1 on next page).

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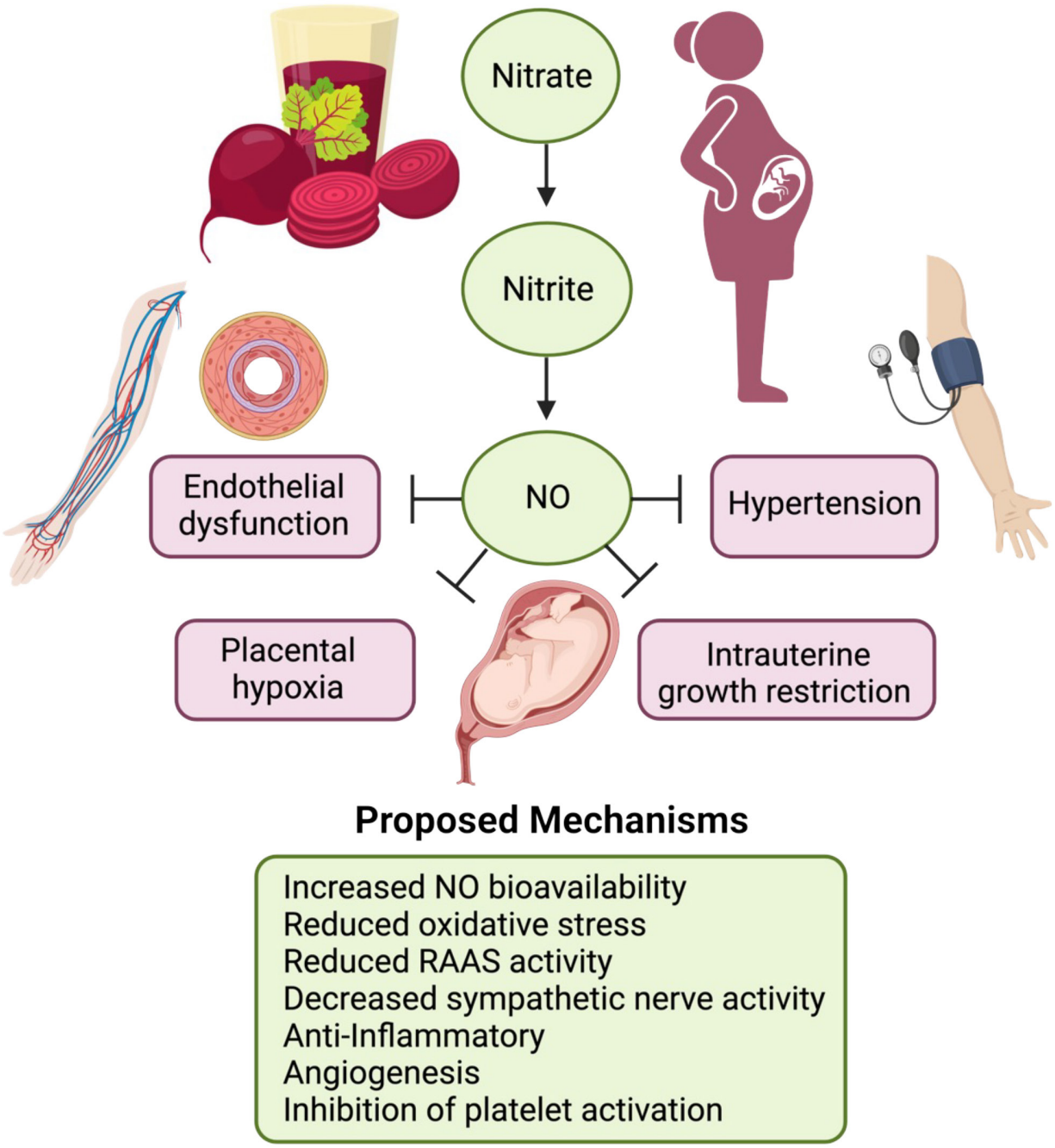


**Figure 1:** Dietary boosting of the nitrate-nitrite-nitric oxide (NO) pathway may have favorable effects in gestational hypertensive disorders including preeclampsia. At least in non-pregnant individuals, intake of nitrate-rich beetroot juice has been demonstrated to improve endothelial function and lower blood pressure. The underlying mechanisms are likely multifactorial and involve different organ systems. In addition to increased production and signaling of NO, lowering of oxidative stress and reduced activity of renin-angiotensin-aldosterone system (RAAS) as well as decreased sympathetic nerve signaling, together with modulation of immune cell function, angiogenesis and platelet function have been demonstrated in clinical and experimental studies.

# LEARNING THE ROPES

## What might Donald Rumsfeld *know* about nitrate, nitrite and hypertension?

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The Rumsfeld Matrix, perhaps an adaption of the earlier Johari Windows, refers to a distillation of what one might know at any one point in time on a subject.[1] We have applied the same framework to our short review on the non-canonical pathway for nitric oxide (NO) generation i.e. the nitrate-nitrite-NO pathway and hypertension. Further more-detailed descriptions of these issues and more are located in our recent review.[2]

**The *known* Knowns**

NO is a key protective element in cardiac and vascular territories and plays a critical role in setting vascular tone and hence is important for blood pressure regulation in health.[3] However, NO bioavailability is diminished in all aspects of the cardiovascular disease continuum and diminished canonical NO production appears to be pathogenic in hypertension and related disorders.[4]

Canonical NO production in the vascular system is triggered within the endothelial cell following stimulation by circulating hormones and shear-stress. This causes rises in intracellular calcium and activation of kinases that mediate endothelial NO synthase activation and phosphorylation leading to oxidation of L-arginine and release of soluble NO. NO in turn binds to and activates the haem moiety on soluble guanylyl cyclase leading to downstream elevation of cyclic guanosine monophosphate and activation of protein kinase G-dependent pathways.[2]

An important characteristic of NO is that it is a free-radical containing soluble, small molecule with ultra-short biological half-life due to its reactive radical nature. Its biological activity in vivo is limited by oxidative reactions particularly with abundant haem-containing proteins (in the circulation oxy-haemoglobin (very fast)) and with molecular oxygen (slow), to produce the relatively stable products nitrate ($NO_3$-) and nitrite ($NO_2$-) respectively.

**The *less-known* Knowns**

Strategies to leverage canonical NO signalling by augmenting endothelial NO synthase activity or directly supplying NO using organic nitrovasodilators have not yielded improvement in cardiovascular outcomes. This is despite therapeutics targeting downstream of NO showing beneficial outcomes, such as soluble guanylyl cyclase activation in pulmonary hypertension, confirming the utility of targeting the pathway. However, an alternative strategy is possible, utilising a lesser-known pathway for NO generation, based on the sequential reduction of nitrate to nitrite and thence NO.

This pathway (variably referenced itself as the alternative, nitrate-nitrite-NO or the more recently used non-canonical NO pathway, see figure 1) requires the initial reductive step to be undertaken in the oral cavity by our facultative symbiotic microbiome.[5] In the absence of these "good" bacteria, nitrate does appear to be

functionally inert in the human cardiovascular system. However, once nitrate is converted into nitrite by the oral microbiome, a plethora of confirmed mammalian nitrite reductases convert nitrite to NO in physiologically relevant conditions and abundance.[6] Nitrite-derived NO leads to elevation of cyclic guanosine monophosphate and recapitulates in pre-clinical models the sine qua non anti-atherogenic, anti-platelet and vasodilatory properties of canonically-produced endothelial NO.

This pathway has been investigated in numerous situations with variable outcomes in exercise physiology, ischaemic syndromes, heart failure but most extensively studied on its effects on blood pressure (see table 1). In humans, both in healthy normotensive volunteers and hypertensive patients, ingestion of inorganic nitrate lowers blood pressure.[7,8] This is evident in both acute and chronic dosing, with no apparent tachyphylaxis.[8,9] Further, this can be achieved with nitrate as a salt (usually sodium or potassium) or in dietary from green-leafy vegetables, such as red beet(root).

**Table 1.** Key publications in development and investigation of non-canonical nitric oxide (NO) generation for blood pressure (BP) lowering.

| Year | Key Experimental Description | Contribution to field | Citation |
|---|---|---|---|
| 1994 | Inorganic nitrate ingestion is associated with increased salivary nitrite levels. Acidification of nitrite generates sufficient NO to kill pathogens (Candida spp, E. Coli). | First description of non-canonical NO production. | Benjamin et al. PMID 8139683 |
| 1994 | Co-incubation of human saliva and dietary nitrate (as lettuce) is associated with NO production in acidic environments. Gastric NO production inhibited by PPIs. | First description of non-canonical NO production. | Lundberg et al. PMID 7828969 |
| 2001 | Nitrite (buffered to pH 6.6) relaxes pre-contracted rat aortic rings; an effect inhibited by soluble guanylyl cyclase inhibition and potentiated by ascorbic acid. | Nitrite acts as a vasodilator in the circulation (in vitro) via soluble guanylyl cyclase. | Modin et al. PMID 11350258 |
| 2003 | Infusion of sodium nitrite into the human forearm causes vasorelaxation with concomitant increased forearm blood flow; a phenomenon augmented by mildly hypoxic conditions formed during exercise. | Nitrite is active in the human circulation as a vasodilator (in vivo). | Cosby et al. PMID 14595407 |
| 2006 | 3 days of sodium nitrate ingestion in healthy volunteers in associated with elevated of circulating nitrite concentration and lowers diastolic BP (~4 mmHg). | First description of BP lowering with inorganic nitrate in healthy volunteers. | Larsen et al. PMID 17192551 |
| 2008 | Acute ingestion of dietary nitrate (~22 mmol, as red beet(root) juice) is associated with maximal BP lowering of ~10/8 mmHg 3h post ingestion, when plasma nitrite concentration is highest. | First description of BP lowering with dietary nitrate in healthy volunteers. | Webb et al. PMID 18250365 |
| 2008 | Interruption of entero-salivary recirculation of nitrate to nitrite in oral cavity, by avoidance of swallowing saliva post dietary nitrate ingestion, prevents elevation of nitrite and prevents BP lowering. | Confirmation of lack of direct bioactivity of nitrate, and importance of oral microbiome. | Webb et al. PMID 18250365 |
| 2013 | Acute ingestion of dietary nitrate (~3.5 mmol as red beet(root) juice) lowers BP (~12 mmHg) and arterial stiffness (pulse wave velocity) in untreated hypertensive patients. | First description of BP lowering in hypertension using non-canonical NO. | Ghosh et al. PMID 23589565 |
| 2013 | Use of antibacterial mouthwash for a week in healthy volunteers is associated with reduction in plasma nitrite that is associated and inversely correlated to BP increase (2-3 mmHg). | First description of circulating nitrite setting basal BP; and potential deleterious effects of oral microbiome disruption. | Kapil et al. PMID 23183324 |
| 2015 | 4 weeks of dietary nitrate (~5 mmol as red beet(root) juice compared to nitrate-deplete placebo juice) lowers by 5-7 mmHg, improves endothelial function and improves arterial stiffness BP in uncontrolled hypertensive patients, with no tachyphylaxis | Dietary nitrate lowers BP in hypertension by almost as much as would be expected by addition of a single extra anti-hypertensive. | Kapil et al. PMID 25421976 |

Important and sustained (for at least 4 weeks) blood pressure (BP) reduction (5-7 mmHg) can be achieved with small volumes (<250mL) of high-nitrate containing vegetable juice.[9] Interruption of the oral conversion of nitrate to nitrite prevents both elevations in measurable circulating nitrite concentrations after nitrate ingestion and prevents blood pressure reductions, confirming the critical role of the oral microbiome in the initial reductive step.[7]

Given that nitrate and nitrite are formed endogenously as oxidative products of NO metabolism, these also undergo interconversion through this pathway under basal conditions. Circulating plasma nitrate and nitrite concentrations are in the order of 20-40 μmol/L and 50-400nmol/L respectively. Interfering with oral nitrate reduction (in the absence of nitrate supplementation) reduces circulating nitrite and is associated with increases (2-3 mmHg) in basal BP.[10]

**The *known* Unknowns**

Whilst it is clear nitrate-derived NO has the same functions as canonical, endothelium-derived NO and that this pathway can be leveraged to lower BP in hypertensive patients, there are several key issues still to be determined.

It is not clear how nitrite is transported in the gastro-intestinal tract once it is swallowed after oral conversion from nitrate. Nitrite enters the acidic stomach environment and rapidly accumulates in the circulation. However, whether there is a distinct nitrite anion-transporter or whether nitrite is protonated to nitrous acid ($HNO_2$) to facilitate trans-membrane transport is not clear. Further, nitrate and nitrite are avidly reabsorbed in the kidney. The molecular machinery for these has not been identified clearly to date though is an area of active research in our laboratory.

There are additionally numerous key nitrite reductases identified, perhaps with the globin family and xanthine oxidoreductase most commonly implicated as key enzymes, though with no consensus as to which is more important under physiological or extreme (i.e. ischaemic) environments. Moreover, in relation to deoxyhaemoglobin-derived NO (the primary globin implicated to date as a nitrite reductase), exactly how NO would escape from metabolism by oxyhaemoglobin, that would be in very close proximity, since it is such an avid reactant remains uncertain.[2]

In fact, it is not universally agreed that nitrite acts only via conversion to NO, with various laboratories providing compelling evidence of nitrated fatty acids, nitrosothiols and direct actions of nitrite being important effectors.[2,11]

Whilst lack of knowledge of the above issues doesn't prevent the measurable function of the non-canonical NO pathway from benefitting the cardiovascular system, there are some more crucial unknowns with respect to BP. There appears to be a reduced effect of nitrate supplementation in patients with diabetes though there is no reasonable explanation to date.[12] Though with how common cardiometabolic abnormalities co-exist, fully understanding the presence and/or degree of diminution of efficacy is important.

More crucially, it has not been established conclusively whether blood pressure lowering with nitrate supplementation is profound enough or long-lasting enough to beneficially reduce hypertensive end-points. The necessary scale of current cardiovascular outcome trials powered to major adverse cardiovascular end-points means this is unachievable for a dietary intervention. It is also not clear whether there are benefits on important surrogate end-points, such as hypertensive heart and vascular damage though these are currently under investigation in our lab (NCT03088514).

**The *unknown* Unknowns**

Of course, in the truest sense, this section has to be empty as we are not able to opine on aspects we don't know that we don't know. But we are sure that there are many!!

**The *unknown* Knowns**

The Slovenian philosopher Slavoj Zizek coined an additional aspect to the Rumsfeld matrix not in the original speech on weapons of mass destruction.[1] The unknown knowns might refer to truths we prefer to ignore or downplay.

In this regard, the unknown known is how do we leverage the above knowledge to help our patients with hypertension, half of whom have uncontrolled hypertension despite the wide availability of cheap, generic medications with decades of exemplary safety and efficacy records. Patients are disinclined to lifelong pharmacotherapy for asymptomatic risk reduction and yet we have mounting evidence of a beneficial dietary intervention that produces appreciable blood pressure reduction but is not used clinically.

Yet without a biotechnology or big pharma company to drive the field, the large studies, the marketing of such an intervention, it doesn't seem that academia alone can provide the quantity of evidence at scale and time-urgency to leverage further the huge gains in scientific knowledge to date. Whether global public health strategies are able to actualise these scientific discoveries is yet to be seen.

It may be that the known unknowns relating to absorption/reabsorption provides other druggable targets that are patentable that could attract significant funding to utilise this pathway. The same approach could be applied once the relative importance in different disease states of the numerous nitrite reductases is incontrovertibly identified and settled.

**Summary**

The related chemicals nitrate, nitrite and NO exist in a cycle of oxidation and reduction in mammalian systems (with the crucial help of prokaryotes). Nitrate supplementation leads to authentic NO production and blood pressure reduction. True clinical translation of these knowns is currently absent.

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# INSTITUTE FOCUS

## Cardiovascular hormone research at the Max-Delbrück Center for Molecular Medicine in Berlin-Buch

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Michael Bader's groups is located at the Max-Delbrück Center for Molecular Medicine in Berlin-Buch (MDC), Germany (Figure 1). The MDC is part of the Helmholtz Association of German Research Centres and performs a program on Systems Medicine and Cardiovascular Diseases. It was founded by Detlev Ganten in 1992 on a scientific campus with historic roots in research on molecular biology, neurobiology, cancer and cardiovascular diseases in the frames of the Kaiser Wilhelm Society and the Academy of Sciences of the GDR. From the very beginning the MDC had a strong focus on translational research and tight links to the Charité-University Medicine Berlin. The mission of the institute has been to perform state-of-the-art basic science to develop novel therapies with one focus on cardiovascular diseases.


**Figure 1:** Max-Delbrück-Center for Molecular Medicine, in Berlin Buch, Germany; buildings from three different eras of its history

### Angiotensin

Michael Bader joined Detlev Ganten's lab in the 1990s pursuing research on cardiovascular hormones in particular on angiotensin (Ang) II (Figure 2). Detlev Ganten had pioneered the use of molecular biology and transgenic animals in cardiovascular research in Germany and had generated the first transgenic rat (TGR(mREN2)27), which was hypertensive due to the overexpression of mouse renin making it a standard model in hypertension research. Follow-up projects created a hypertensive double-transgenic rat with the human renin angiotensin system (RAS) (see PNAS 1992) which was used to develop renin inhibitors and turned out to be a suitable model for preeclampsia, and an animal with reduced AngII production in the central nervous system, which was instrumental to functionally characterize the brain RAS (PNAS 1999). Transgenic mice overexpressing angiotensinogen in the whole body or only in the brain (Clin Sci 2021) followed and confirmed the importance of local RAS in different tissues. The first knockout mouse produced in the Bader lab carried a mutated MAS

**Figure 3:** Michael Bader, Natalia Alenina, Enno Klussmann, Robson Santos, and Joao Pesquero.

protooncogene which at this time was thought to be an AngII receptor (JBC 1998). In collaboration with Thomas Walther in Berlin and Robson Santos (Figure 3) in Belo Horizonte (Brazil) and using these mice, it could be shown that Mas is, in fact, the receptor for Ang1-7 (PNAS 2003). The enzyme mainly responsible for the generation of this metabolite of AngII is angiotensin-converting enzyme 2 (ACE2) (Figure 2) and therefore the Bader group, in collaboration with Robson Santos, Maria-José Campagnole-Santos also from Belo Horizonte and Luiza Rabelo from Maceio (Brazil), started to work on Mas, Ang1-7 and ACE2 by generating and analyzing transgenic and knockout animal models for these factors. Animals overexpressing these molecules were in general protected from cardiovascular diseases and knockout mice showed deleterious effects on cardiovascular organs (Physiol Rev 2018). These findings contributed to the novel concept of a protective axis of the RAS consisting of ACE2, Mas and Ang1-7. Very recently, animals overexpressing human ACE2 became again instrumental to study

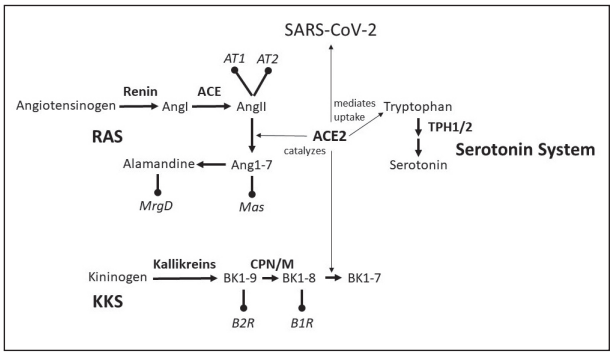


**Figure 2:** Cardiovascular hormone systems studied by the Bader group, with ACE2 as bridging factor between them. RAS, renin-angiotensin system; KKS, kallikrein-kinin system; BK, bradykinin; Ang, angiotensin; CP, carboxypeptidase; ACE, angiotensin-converting enzyme; TPH, tryptophan hydroxylase; receptors are shown in italics, enzymes in bold.

the novel notorious function of ACE2, being the receptor for SARS-CoV-2, the virus causing COVID-19. The Bader group also contributed to the discovery by Robson Santos of another protective RAS peptide, alamandine, which is Ang1-7 with an N-terminal alanine binding to its own receptor, MrgD (Circ Res 2013).

## Bradykinin

In parallel to the work on the RAS, the group started to work on the kallikrein-kinin system (KKS) in close collaboration with Joao Pesquero from Sao Paulo (Brazil). First, genes for both kinin receptors, B1R and B2R, were cloned (JBC 1994, BBRC 1996) and knockout mice were generated for B1R (PNAS 2000) and for both receptors (FASEB J 2007). Surprisingly, the latter animals were completely normal revealing that the KKS is not essential for the life of a mouse. Nevertheless, in collaboration with numerous other groups the Bader group revealed important functions particularly of the B1R in the pathogenesis mostly of inflammatory diseases using these animal models. Moreover, transgenic rats overexpressing tissue kallikrein thereby mainly activating B2R exhibited protective actions on cardiovascular organs (FASEB J 2000). Thus, kinin receptors became valid drug targets which are not yet sufficiently exploited.

## Serotonin

In the early 2000s researchers from the Charité approached Michael Bader, suggesting to knockout tryptophan hydroxylase (TPH) the at this time only known rate-limiting enzyme in serotonin synthesis. Since besides its most famous role as neurotransmitter, serotonin is mainly a peripheral vasoactive hormone generated in the gut and transported and released from platelets

in the blood, such animals were also of interest for a cardiovascular hormone research group. The resulting TPH knockout mice had very low, but still detectable levels of serotonin in the blood and surprisingly completely normal levels in the brain (Science 2003). An intensive screen of the just published human genome discovered a homologous gene to TPH, which was cloned and proven to encode a second TPH protein in all vertebrates, called TPH2 in contrast to TPH1, the renamed already known enzyme. Of note, the Bader group could recently show using phenylalanine hydroxylase knockout mice that this enzyme is responsible for the generation of the residual serotonin in the blood of TPH1-deficient mice (FASEB J 2021). TPH2-knockout mice were generated by Natalia Alenina in the Bader lab and exhibited, as expected, normal levels of serotonin in the blood but completely lacked the monoamine in the brain (PNAS 2009) again supporting the concept of a blood/brain duality of the serotonin system. These animals were surprisingly viable but showed several abnormalities in growth and behaviour. TPH1 knockout mice were used to study the peripheral functions of serotonin again in collaboration with numerous other groups worldwide. These studies revealed that the animals were protected from several diseases including thrombosis and pulmonary hypertension, and discovered that serotonin can not only act via receptors on the cell membrane but also by being covalently linked to proteins in the cytosol, a process called serotonylation (Cell 2003, Circulation 2008, Nature 2019). Based on these findings the Bader lab initiated a drug screening project to find TPH1 inhibitors for the treatment of these diseases. Together with Edgar Specker, Jens von Kries and Marc Nazaré, they screened a compound library at the Research Institute for Molecular Pharmacology on the Berlin-Buch campus and found a novel class of TPH inhibitors (J Med Chem 2022) which were chemically optimized and successfully tested in preclinical disease models. Now a spin-off company, TRYPTO Therapeutics, is in foundation which will initiate clinical trials to get these compounds into the clinic at first for the treatment of pulmonary hypertension. Thereby, the TPH inhibitor project became a role model for all projects in the Bader group and in the MDC, going from the early discovery of a novel drug target in genetically modified animal models followed by the screening and development of suitable compounds and their validation in preclinical disease models eventually to the clinical evaluation of new therapeutic options. Comparable projects are on the way in the Bader group targeting ACE2. Interestingly this protein turned out to bridge all three hormone systems studied in the lab (Figure 2). It was already known to transform AngII to Ang1-7 and degrade kinin B1R agonists when the Bader lab discovered that its essential function in intestinal tryptophan uptake also leads to a marked reduction of the tryptophan metabolite serotonin in the blood and in the brain of ACE2-knockout mice (Cell Mol Life Sci 2018).

**PDE3A**

With the advent of gene-targeting technology for the rat the Bader group also started to generate knockout and knockin rat models to further exploit the advantages of this species compared to the mouse in cardiovascular research. In collaboration with Enno Klussmann and Friedrich Luft at the MDC, knockout and knockin rat models were established with mutations in the gene for phosphodiesterase 3A (PDE3A), which confirmed the causative role of this protein for the rare genetic disease Hypertension with Brachydactyly but also showed that its activity protects the heart from hypertensive damage (Circulation 2020, 2022). Interestingly, PDE3A had popped up in several genome-wide association studies as candidate gene for essential hypertension making it a novel antihypertensive drug target and the genetically modified rats may be suitable models to develop corresponding therapies.

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# ISH MATTERS

## ISH New Council Members 2022-2026

**The International Society of Hypertension (ISH) was pleased to welcome its newly elected Council Members during the Gala Dinner at the ISH Kyoto 2022 Meeting on 15th October 2022.**

Brief biographies of the three individuals who will serve an initial four year term (2022-2026) are provided below.

### Tazeen Jafar (Singapore)

Dr Tazeen H Jafar is a clinician (nephrologist)-scientist renowned globally for expertise and experience in implementation research in hypertension and clinical risk prediction in cardiovascular disease and chronic kidney disease across various settings. She is a tenured Professor in the Program in Health Services and Systems Research at Duke-NUS Medical School, Singapore, Visiting Consultant Renal Medicine at Singapore General Hospital, Visiting Professor of Medicine, Aga Khan University, Karachi, Pakistan, Consultant nephrologist, Durham VA Hospital, and Research Professor at Duke Global Health Institute, Durham, NC, USA.

### Kazuomi Kario (Japan)

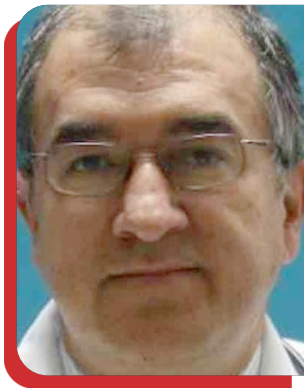
After graduating from Jichi Medical University in 1987, Professor Kario worked at Awaji City National Health Insurance Hokudan Clinic, the Cardiovascular Center at Cornell University Medical School in the U.S., was a visiting professor at Columbia University School of Medicine, and a COE professor at Jichi Medical University. His current positions are as follows: Professor and Chairman, Jichi Medical University School of Medicine, Japan; adjunct professor, Yonsei University School of Medicine, Korea; and distinguished professor, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, China. He is currently vice president of the Japanese Society of Hypertension, a councillor of the Japanese Society of Cardiology, a fellow of the American College of Cardiology, the American Heart Association, and the European Society of Cardiology.

### Cesar Romero (USA)

Cesar Romero is an Argentinian physician and scientist dedicated to the study of hypertension and its mechanisms. Dr Romero received his MD and PhD from the National University of Cordoba, Argentina. As a medical student and early in his career as a physician, he was mentored by the nephrologist Marcelo Orias, MD in the study of genetic and hemodynamic characteristics in young hypertensive patients. After his residency, he worked as attending physician and clinical investigator in Argentina, where he still coordinates a hypertension research group. He has postgraduate experience in Brazil under the supervision of Dr Muxfeldt (Rio de Janeiro) and Dr Latini (Santa Catharina, Brazil); in Detroit, Michigan under the supervision of Oscar Carretero, MD; and in Atlanta, Georgia under supervision of Dr Susan Wall. Currently, he is assistant professor at Emory University in Atlanta, USA.

# ISH MATTERS

## Colombia and the 2024 ISH meeting: Improving the control of hypertension worldwide



PATRICIO LOPEZ-JARAMILLO
DAGNOVAR ARISTIZABAL
Sociedad Colombiana de Cardiología and
International Society of Hypertension

**Introduction**

Cardiovascular disease (CVD) is the leading cause of death globally, accounting for approximately 32% of all deaths, with approximately 33% of which occurred prematurely. Although the prevalence of CVD is rising globally, developing regions have both a higher incidence rate and cardiovascular mortality. In low- and middle-income countries (LICs and MICs) CVDs are contributing to poverty due to catastrophic health expenses borne by patients and premature deaths during their most productive years, with wide-scale effects on the economy.[1] Large-scale prospective cohort studies have shown that about 70% of CVD cases can be attributed to a small cluster of individual modifiable risk factors, among which hypertension, high non-high-density lipoprotein (HDL) cholesterol, low education, tobacco use, household air pollution, and poor diet have the biggest population attributable fractions (PAFs).[2]

Hypertension (HTA) remains as the principal risk factor for CVD worldwide, particularly in LICs and MICs. In a recent study in South American countries[3] we have demonstrated that for CVD, largest PAFs were due to hypertension (18.7%), abdominal obesity (15.4%), tobacco use (13.5%), low strength (5.6%), and diabetes (5.3%). For death, the largest PAFs were from tobacco use (14.4%), hypertension (12.0%), low education (10.5%), abdominal obesity (9.7%), and diabetes (5.5%) [figure 1].

Despite of the important contribution of HTA to CVD and mortality, the rates of awareness, treatment and control are so low globally but particularly in LICs and MICs. The PURE study shows that among the 142 042 participants 40.8% had hypertension but only 46.5% were aware of the diagnosis.[4] Of those who were aware of the diagnosis, the majority (87.5%) of those who were aware receiving pharmacological treatments, but only a minority of those receiving treatment were controlled 32.5%. Overall, 30.8% of treated patients were taking 2 or more types of blood pressure–lowering medications. The percentages aware, treated and controlled in LICs were lower in LICs compared with all other countries.

These findings suggest substantial room for improvement in hypertension diagnosis and treatment. This is the reason why in our next meeting of the ISH one of the principal aims will be a deep discussion about the strategies to improve the control of hypertension worldwide. We will have the opportunity to present the results of the MMM initiative in the specific goal of improve hypertension awareness[5], and how the contribution of clinical trials as HOPE-3[6], HOPE-4[7], programs as RESOLVE and HEARTS[8], and the new guidelines of the ISH[9] and WHO[10] could be implemented globally to improve the rates of diagnosis, treatment and control of hypertension.

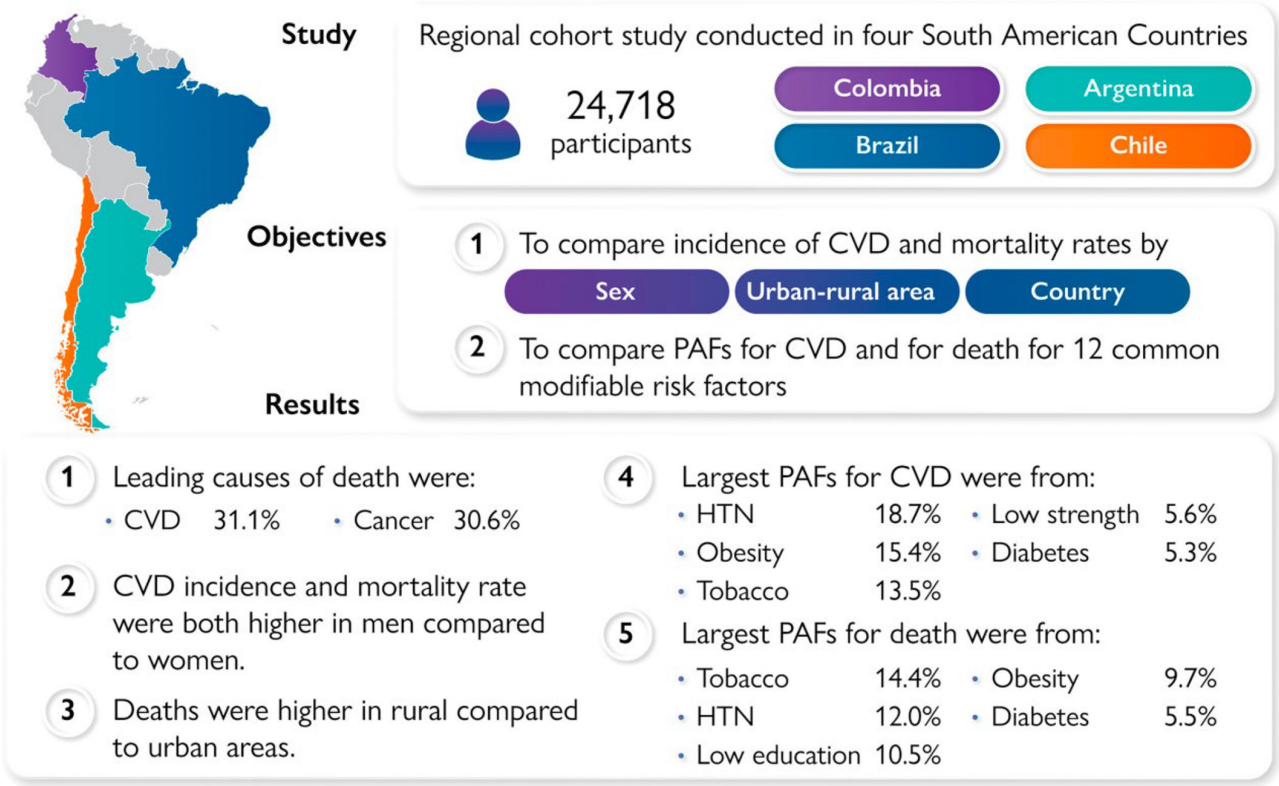



**Figure 1.** Cardiovascular diseases (CVD) are the leading cause of mortality in South America and hypertension have the largest population attributable fraction for CVD. [Reproduced of reference 3].

The next Congress of the International Society of Hypertension in 2024 will be held in Cartagena, Colombia, one of the most beautiful colonial cities in Latin America. Cartagena has been declared world's heritage site since 1984, due to its amazing architecture and history. Cartagena´s ensemble of forts is not only the biggest in South America, but also one of the best preserved; in addition, its coastal location and charming cuisine, has made the city an highly attractive tourist destination. More than one million tourists from around the world visit Cartagena every year.[11]

It's been a long time since our ISH Congress was in Latin-America. For this come back we have already started our journey to make your visit a memorable scientific and cultural experience. A plural regional organizing committee linked with all cardiovascular and hypertension societies in Latina America will be involved in this undertaking. Scientific societies such as Pan-American Health Organization, World Heart Federation, Latin-American Society of Hypertension and Interamerican Society of Cardiology have endorsed the ISH 2024.

We look forward to seeing all the delegates from around the world, dedicated to research, diagnose and treat high blood pressure in Cartagena. In addition to discuss the best strategies to improve the control of HTA, our ISH 2024 Congress will be focused on four key topics:

**1. Hypertension care with multidisciplinary teams and standardized processes, we will also explore the application of telemedicine and the new remote patient monitoring techniques and devices.**

**2. Predictive and analytical models of response to non-pharmacological and pharmacological therapies.**

**3. Evaluation and treatment of special populations: elderly, ethnic groups, minorities, frail and multi-morbid patients.**

**4. Applied research and translational medicine: molecular and pathophysiological mechanisms, genetic factors and hypertension in animal models.**

In addition, several inter-society symposiums will be held, which will be a great opportunity to interact and make connections with eminent experts and leaders of arterial hypertension from around the world. The fact that the ISH Congress have become such a great gathering to display and discuss the research developments in hypertension has helped everyone to have a better understanding of the rapid changes in in the field and to focus the research efforts accordingly. We, as a part of the Organizing Committee, are certain that the takeaways of the 30th Congress of the ISH will further deepen our understanding to improve the control of hypertension worldwide.

We are sure that Cartagena will love you and we are doing the best to guarantee that your stay in Colombia will be full of science, joy and happiness

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# ISH MATTERS
# ISH 2022 Award Winners

**We are delighted to announce the winners of the ISH 2022 awards who were presented during the ISH Awards and Lectures Session on 15th October in Kyoto, Japan.**

## ISH Franz Volhard Award and Lectureship for Outstanding Research

This Award and Lectureship was endowed by Farbwerke Hoechst in 1972 to commemorate the centenary of the birth of Franz Volhard. The award is made biennially to a person or persons who has initiated in the field of hypertension or in a related discipline, a concept which remains of current interest. The recipient shall be invited to deliver to the ISH a lecture on the topic for which the award is bestowed.

### Recipient: Suzanne Oparil, USA

The Awards Committee recognised Dr Oparil's outstanding contributions to basic clinical and population cardiovascular research that shaped medical practice in hypertension. Dr Oparil has made significant contributions to AHA, ASH, and American Federation for Clinical Research and indeed ISH in leading positions. She has held important advisory positions with the National Institutes of Health and led Task Forces, expert panels, peer-review and guidelines committees, served as Co-chair of the Joint National Committee charged with developing the JNC 8 U.S. guidelines for the prevention, detection, evaluation and treatment of high blood pressure. She has received numerous honorary memberships, lectureships and distinguished awards for her contributions to academic medicine. The Society is grateful for the immense impact of Dr Oparil's contribution to research and education on management of hypertension around the globe.


Dr. Oparil gave a lecture at the ISH Kyoto meeting entitled: "My Road in Academic Cardiology"

# ISH Robert Tigerstedt Lifetime Achievement Award

This award was originally endowed by Merck Sharp & Dohme International in 1974. The award is presented biennially, on the recommendation of the ISH Awards Committee to a person, persons or institution responsible for distinguished work relating to the aetiology, epidemiology, pathology or treatment of high blood pressure. The achievements of the recipient should reflect distinguished contributions in research, teaching or clinical activities relating to the aetiology, epidemiology, pathology or treatment of high blood pressure and successful mentorship of younger colleagues.

**Recipients:**

A number of extremely high calibre nominations were received for this award this year. The Awards Committee decided to recognise two world-leading scientists for the immense impact of their research in hypertension

## Anna Dominiczak, UK

The Awards Committee has appreciated Professor Dame Anna Dominiczak's lifetime contributions to hypertension research and clinical practice. Since obtaining her first medical degree in 1978, she has made substantial and novel contributions to advance research in hypertension, cardiovascular genomics and precision medicine where she led major research programmes. Apart from contributions to research, she has made a major impact on clinical practice and education as President of the Association of Physicians of Great Britain and Ireland (2018-2021), President of European Society of Hypertension and Secretary of ISH and Editor-in-Chief of Hypertension. She has supervised and mentored numerous researchers in the field of hypertension many of whom are in top leadership positions in academia. The Society is grateful to Professor Dame Anna Dominiczak for accepting the ISH Robert Tigerstedt Award.

## Eoin O'Brien, Ireland

The Awards Committee was impressed by Professor O'Brien's outstanding lifetime contributions to hypertension research and clinical practice. He is widely acknowledged by his international colleagues to have made an extraordinary contribution to the blood pressure (BP) measurement field with enormous influence globally. Professor O'Brien has published over 800 scientific papers on BP measurement methodology and technology that have shaped clinical practice and influenced international guidelines. Professor O'Brien led the development of the BHS and ESH-IP protocols for the validation of BP monitors and founded the first international initiative to evaluate the accuracy of BP monitors to provide guidance to healthcare professionals and patients. The Society is grateful to Professor O'Brien for accepting the ISH Robert Tigerstedt Award.

## ISH Developing World Award

This award is for a researcher or researchers in the developing world who has done outstanding work in the region.

### Recipient: Andre-Pascal Kengne, South Africa

The Awards Committee recognized Profesor Kengne's excellent contributions to hypertension research and clinical practice and outstanding work in the developing world with a focus on Africa. The Society is grateful to Professor Kengne for accepting the ISH Developing World Award.

## ISH Paul Korner Award

**Supported by the High Blood Pressure Research Foundation**

This award was endowed by the High Blood Pressure Research Foundation in 2013 to honour the late Paul Korner, an internationally renowned expert in the field of hypertension with a special interest in the neuroscience of blood pressure. The award is presented to a person who has demonstrated outstanding contributions to research on hypertension in the broad field of neuroscience.

### Recipient: Daniela Carnevale, Italy

## ISH Distinguished Fellow Award

Members who have given outstanding service to the ISH and have made unusually distinguished contributions to experimental and clinical research in hypertension may be nominated to be distinguished Members of the Society.

### Recipient: Sverre Kjeldsen, Norway

## ISH Award of Excellence for Research in Cardiovascular Health and Disease in Women

The ISH Women in Hypertension Research Committee established this award in 2018 to recognise and encourage research in cardiovascular health and disease in women.

**Recipient: Heddwen Brooks, USA**

## ISH Honour for Senior Women Researchers

ISH women members who have given outstanding service to the ISH and / or made exceptionally distinguished contributions to experimental and clinical research in hypertension may be nominated to receive an ISH Honour for Senior Women Researchers. This award was introduced in 2016 by the ISH Women in Hypertension Research Committee.

**Recipients:**

Award winners this year come from all corners of the globe.

**Ibtisam Ahmed Ali Babiker (Sudan)**

**Yook-Chin Chia, Malaysia**

**Stephanie Watts, USA**

## ISH Mid-Career Award for Women Researchers

The ISH Women in Hypertension Research Committee established this award in 2018 to recognise, encourage and inspire women in science and medicine in the field of hypertension and related cardiovascular diseases.

### Recipient: Meena Madhur, USA

## ISH Austin Doyle Award

This award marks the contribution of Austin Doyle, Past President of the ISH and Founding Chairman of the High Blood Pressure Research Council of Australia. It is awarded to a graduate, who is within 5 years of post-graduate qualification.

### Recipient: Dean Picone, Australia

**Abstract presented:**

Prevalence of validated blood pressure measuring devices being sold by Amazon: 12-month prospective analysis across 10 countries

## ISH New Investigator Oral Presentation Awards

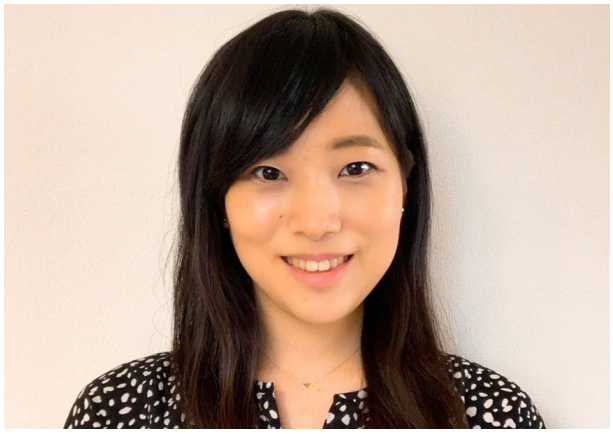
This award was established in 2012 to encourage New Investigators and recognise excellence in scientific contribution at the ISH Biennial Scientific Meetings and will be awarded to New Investigators judged from a group of finalists to have given the best oral presentations.

### Winner: Chia-Te Liao, Taiwan / Belgium

**Abstract presented:**

Cost-effectiveness of Intensive versus Standard Blood-Pressure Control among hypertensive patients in Taiwan: A simulation modelling study

### Runner up: Yoko Inagaki, USA



**Abstract presented:**

May Measurement Month 2021: An analysis of blood pressure screening results from Nepal.

# AFRICAN VOICES

## Introduction

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DOI: 10.30824/2212-17

In the previous issues of Hypertension News, the African Voices section featured research on the prevalence of hypertension and associated risk factors, the role of task shifting and multicomponent strategies in improving hypertension management and the blood pressure lowering effects of indigenous medicinal plants. In this issue, we present two papers, one covering discovery work linked to the renin angiotensin aldosterone system (RAAS) and another focusing on physiological determinants of hypertension in Africans.

The RAAS cascade remains a major source of interest in cardiovascular research owing to its role in cardiovascular disease development and progression and the consequent cardio-renal protection derived from therapeutic agents targeting the system. Of particular interest in an African context is the low RAAS activity and potential link to salt-sensitive hypertension and target organ damage. This issue of Hypertension News features some ground-breaking discovery research on angiotensin converting enzyme (ACE) and new observations on the potential role of increases in systemic flow to hypertension in Africans.

Firstly, Lizelle Lubbe and colleagues from the University of Cape Town, South Africa, discovered first structures of the complete ACE protein by following a different approach from the methods used in previous studies investigating structures of the ACE protein. In this study, cryo-electron microscopy (cryo-EM) was used to determine holistic first structures of the ACE protein. This discovery enhances the understanding of the physiological effects of ACE dimerization and has important implications for potential development of novel ACE inhibitor drugs.

Moving to population studies, Keneilwe Mmopi from the University of Botswana in collaboration with the University of the Witwatersrand gives a summary of the body of work on the determinants of hypertension in Africans. This paper elaborates on, among others, the potential role of age-related increases in systemic flow to hypertension in an African population residing in Soweto. One of the key observations is the dissociation between salt-sensitivity and renal mechanisms of hypertension in this population, with systemic flow being the dominant determinant of hypertension with a strong hereditary component.

Indeed, the RAAS continues to be a target for development of novel antihypertensive treatment as supported by the current discovery of complete structural components of the ACE protein. The phenomenon of salt sensitivity as a contributing factor to hypertension in populations of African ancestry remains a relevant subject in cardiovascular research. More studies are needed to contribute to the understanding of the pathophysiological mechanisms leading to elevated blood pressure and development of tailored treatment options for populations of African ancestry.

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# AFRICAN VOICES

## Advanced microscopy provides structural insight into a highly dynamic hypertension target

LIZELLE LUBBE

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DOI: 10.30824/2212-18

Angiotensin converting enzyme (ACE) is one of the key targets of current antihypertensive therapy with ACE inhibitors being one of the four recommended first-line therapies[1]. In some patients, however, ACE inhibitors trigger adverse effects such as persistent cough (5-20%)[2] or angioedema (0.1-0.7%)[3]. Current ACE inhibitors non-selectively block both domains of ACE and thereby lead to bradykinin accumulation and angioedema. The two domains of ACE are both catalytically active but differ in physiological function with the C- and N-terminal domains respectively responsible for blood pressure regulation, and cleavage of the antifibrotic peptide Ac-SDKP. C- or N-domain-selective ACE inhibitors could thus allow for safe and effective reductions in blood pressure or fibrosis, respectively. To date, however, the development of such compounds has partly been hampered by a poor understanding of the structure of the full enzyme.

Prior to our study[4], the only ACE structures available were of either the N- or the C-terminal domain in isolation (Figure 1a). Furthermore, the majority of glycans on the protein's surface were removed to allow structural elucidation by X-ray crystallography. Our understanding of the structure, function, and inhibition of ACE was thus based on its appearance in an unnatural state[5]. While it was useful for examining the detailed drug interactions in the active site, it fell short on providing functional insight for the native protein. Inhibitor-induced ACE dimerization, for example, occurs and results in intracellular signalling – an event that could not be understood from the truncated single-domain crystal structures. Obtaining functional insights like the mechanism of interdomain interactions is especially important given the growing diversity of ACE-related biological processes and the known occurrence of cooperativity upon ligand binding to ACE[5].

We therefore followed a different approach to previous structural studies and determined the structure of full-length, glycosylated ACE using cryo-electron microscopy (cryo-EM)[4]. With this Nobel prize-winning technique, the protein is rapidly frozen in a thin film of amorphous ice and images of the immobilized protein in different orientations are captured and reconstructed into a 3D model. Since this does not require crystallization, a holistic and physiologically relevant view of the native apo ACE was obtained in our study[4]. While the majority of ACE was monomeric, a fraction of the protein was dimeric despite the absence of inhibitor. This allowed 3D reconstruction of both forms of ACE from a single dataset. By rapidly freezing the protein, its different conformational states were captured and furthermore allowed us to solve molecular movies of how monomeric and dimeric ACE move. Our study thereby provided several important insights.

Monomeric ACE is hourglass-shaped with the homologous N- and C-terminal domains separated by a short linker loop which wraps around the protein surface (Figure 1b). This loop was highly mobile and mediated communication between the domains through bending, pivoting, swinging, and jumping motions. This was accompanied by opening and closing of the active site clefts. Importantly, the linker loop on the protein surface is closely associated with key substrate-anchoring loops which traverse from the surface to the active site core. Dimerization occurred near these loops (Figure 1c) and appeared to trigger ACE inactivation

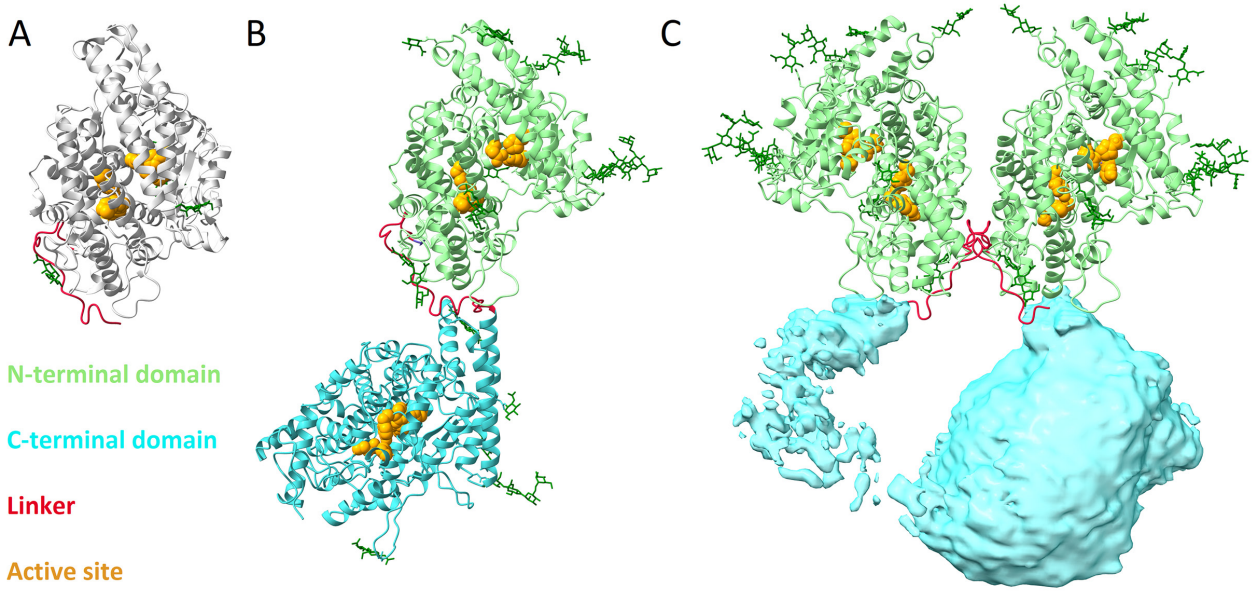


**Figure 1**

Cryo-EM revealed the structure of ACE in two different conformations. (A) The previously published X-ray crystal structure of the truncated N-terminal domain of ACE (PDB ID 3NXQ) could not provide insight into the full-length protein structure and function; the cryo-EM structures of the full-length ACE (B) monomer and (C) dimer as described in our study 4 offered a holistic, physiologically relevant view of the protein.

since the protein's dynamics and the conformation of critical catalytic residues in the active site were altered. In agreement with this hypothesis, our computational analysis of the monomeric ACE dynamics identified a surface pocket formed by these loops as an allosteric site.

In summary, while previous X-ray crystal structures offered partial insight into the structure and function of ACE, we have used cryo-EM to determine the first structures of the complete ACE protein. The findings from our study have important implications for the design of domain-selective ACE inhibitors with better safety profiles since it enhanced our understanding of the protein's function, and allowed visualization of interdomain communication and identification of allosteric sites which could lead to the design of a novel class of ACE inhibitors. Additionally, elucidation of the dimerization interface will now allow further research into the physiological effects of ACE dimerization.

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# AFRICAN VOICES

## Contribution of systemic blood flow to hypertension in Africa

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Of all deaths caused by cardiovascular disease, 80% occur in low-to-middle income countries. In Africa, the dominant risk factor is unquestionably, hypertension. Hypertension is more prevalent and often associated with worse target organ damage in groups of African descent. Hypertension in those of African ancestry is frequently a low-renin form, possibly mediated by a genetic predisposition to salt sensitivity. This genetic predisposition is thought to reflect a greater ability of ancestors of those of African descent, to survive the harsh conditions of slave ships, or an evolutionary change which enabled survival in hot, arid, equatorial environments. Although salt-sensitivity has previously been attributed to renal mechanisms, contemporary thought has challenged this notion. Alternative hypotheses include abnormalities in the handling of salt in subcutaneous tissue. These arguments originated from the evidence that salt-sensitivity is a vascular response to salt loading, with little evidence for even a modest increase in systemic flow. Some evidence for increases in systemic flow would support a vascular autoregulatory response to renal-related increases in systemic flow. However, our group have now provided the evidence that a persistent increase in systemic flow does occur in Africa and that this is a fundamental determinant of hypertension across the adult age.

In a large randomly selected community-based study conducted in SOWETO, South Africa, we have demonstrated that aged-related increases in systemic flow (stroke volume, cardiac output, and peak aortic flow) are primary determinants of hypertension across the full adult age range.[1] This translated into flow-dependent mechanisms in both isolated systolic hypertension as well as systolic-diastolic hypertension and the contribution of systemic flow was as strong as the vascular determinants of blood pressure (BP).[2] Importantly, in the same year we demonstrated that relationships between salt intake (24-hour urinary Na+/K+) and BP in this community were explained by associations between salt intake and aortic characteristic impedance (Zc) and not systemic flow.[3] However, age-related increases in systemic flow and not Zc, systemic vascular resistance (SVR) or arterial compliance were strongly associated with fractional Na+ excretion and creatinine clearance.[4] Of note, these relationships are the strongest BP (and additional confounders)-independent relationships noted between renal function and the hemodynamic determinants of BP ever reported on. These data therefore suggest that a dissociation between salt-sensitivity and renal mechanisms of hypertension exists in this population. Furthermore, consistent with a genetic predisposition to fluid retention, measures of systemic flow show marked intrafamilial aggregation and heritability, while Zc and SVR show modest heritability only.[5]

There are no antihypertensives, including diuretic agents, that are able to decrease systemic flow for prolonged periods without the values returning

to pre-treatment levels given a sufficiently long enough period of time to do so. Therefore these data beg several questions. First, as current therapy can decrease BP in Africa, the question arise as to whether it is important to decrease systemic flow, or is a reduction in SVR with current therapy sufficient? In this regard, our group have now identified the presence of increases in systemic flow in resistant hypertension and several possible BP-independent end organ effects of increases in systemic flow including increases in aortic stiffness caused by aortic distension, and a possible impact on glomerular hyper-filtration. Second, if targeting increases in systemic flow is necessary, how may this be achieved? In this regard, as the impact of diuretics on systemic flow has never been assessed in flow-dependent hypertension, we are assessing this question in the present community. In addition we have in-part identified some of the humoral mechanism involved. In this regard, an increase in circulating aldosterone concentration occurs despite a reduced renin release (caused by the increased systemic flow).[1] Moreover, an attenuation of atrial natriuretic peptide release is also noted in response to the volume overload.[6] In short, the work from our group has provided the first strong evidence to support an age-related, genetically renal-dependent increase in systemic flow as a cause of primary hypertension. Notably this occurs in an African population whose ancestors originated from equatorial regions. Of significance, this effect dissociates from the impact of salt intake on BP. Whether targeting an increase in systemic flow is necessary and the approach to achieve this effect, is presently under investigation.

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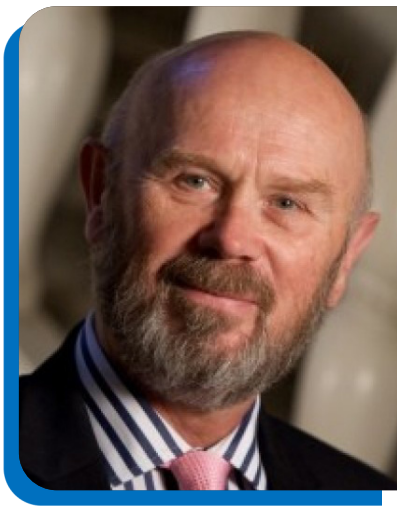
# MMM REPORT

## May Measurement Month adds new supplement to its publications



NEIL POULTER

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The 2022 campaign of May Measurement Month (MMM), the annual global screening campaign that helps to raise the importance of blood pressure (BP) measurement, has closed having once again extended the screening period from May to Autumn. The data from 2022 are currently being collated and cleaned in preparation for analyses to take place as soon as possible to allow submission to a journal in early 2023.

The aims of MMM have remained consistent from the start—to raise awareness of the importance of the measurement of BP at the individual and population level and to provide a temporary pragmatic solution to the shortfall in BP screening programmes in countries around the world. Since its initiation in 2017, over five million volunteers from more than 100 countries have had their BPs screened.

Each year the global results of the annual MMM campaigns are published in a major cardiovascular journal. The MMM 2021 results (delayed significantly due to COVID-19) are currently being reviewed by one such journal and include a global reflection of the impact of COVID-19 on BP measurement and management. Meanwhile, we have now produced four supplements in the European Heart Journal Supplements the first three of which were compilations of the national data produced each year. A minimum number of screenees of 2500 was required for each national paper to be included in these supplements in order to ensure that the analyses carried out, generated reasonably valid point estimates for each year.

This year we have produced a smaller supplement which allowed 12 countries which hitherto had not published their national data to either consolidate their data collected over more than one campaign to produce a significantly large sample, or report on previous annual data of significant size having not done so in the past. These 12 countries were invited to publish a short report of their previously

unpublished MMM data from 2017 to 2019 and the supplement that draws these manuscripts together has just been published.

By virtue of the large numbers of screenees participating each year and the global coverage of the campaign, MMM potentially provides a unique research platform, to allow sub-studies to be carried out. MMM's collaboration with AF-SCREEN this year recruited countries to begin atrial fibrillation screenings alongside BP readings. This sub-study will include results of a pilot run in 15 countries including Australia, China, Georgia, Nepal, Portugal and Poland. We plan to extend this combined BP/Atrial fibrillation screening again in MMM 2023. A further sub-study involves an evaluation of the association between air pollution and BP levels at the ecological and individual level. Reports of these studies are in preparation for submission to the relevant sponsors and medical journals.

Prof. Neil Poulter, CI of the MMM Campaign said

***"We're thrilled with the take-up from countries participating in MMM 2022 despite the shadow of COVID-19 still very real in parts of the world. The introduction this year, and beyond, of atrial fibrillation screening and work on pollution shows that MMM is generating unique data whilst raising awareness of the biggest contributor to global morbidity and mortality."***

For more information about how you can support, visit www.maymeasure.org

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