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Novel diagnostic methods and potential treatments in cardiovascular autonomic dysfunction

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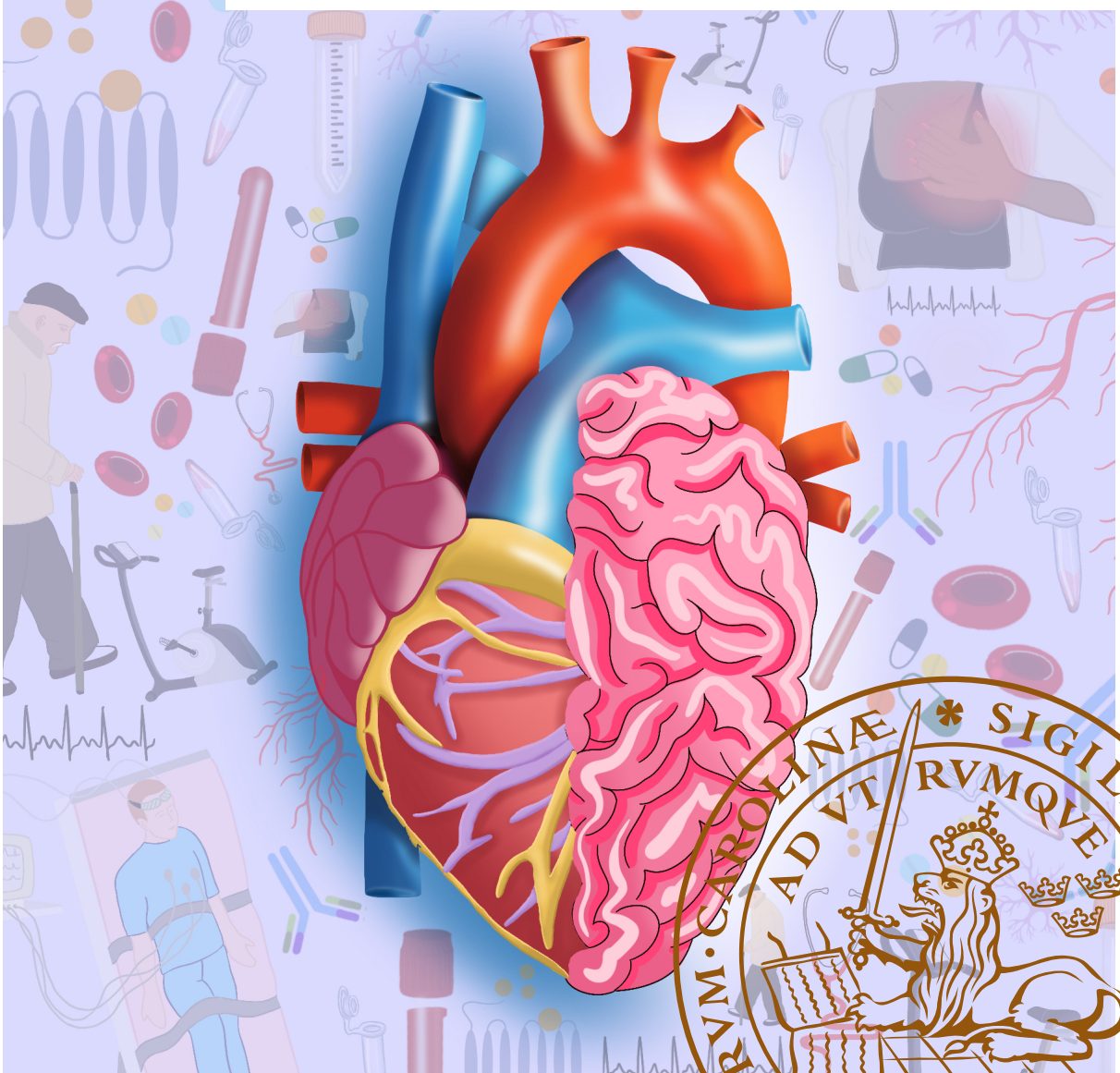

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ISABELLA KHARRAZIHA is an MD and resident in cardiology at Skåne University Hospital in Malmö, Sweden. In parallel to clinical work, she has been researching cardiovascular autonomic dysfunction for several years. Isabella aims to enhance the understanding and management of complex conditions of cardiovascular autonomic dysfunction by exploring novel diagnostic tools and potential treatment options.

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MD

LUND
UNIVERSITY

DOCTORAL DISSERTATION
Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on Friday, September 27, 2024, at 09.00, in Kvinnoklinikens aula, Skåne University Hospital, Malmö.

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Title and subtitle: Novel diagnostic methods and potential treatments in cardiovascular autonomic dysfunction

Abstract

General Aim: To investigate novel diagnostic tools and treatment options for cardiovascular autonomic dysfunction (CVAD) patients, focusing on syncope, orthostatic intolerance, and heart failure (HF).

Background: CVAD includes common clinical entities such as vasovagal syncope (VVS) postural orthostatic tachycardia syndrome (POTS) and orthostatic hypotension (OH). Also, CVAD plays an important role in HF. Monitoring of cerebral tissue oxygenation (SctO2) during orthostasis may aid in understanding mechanisms in CVAD. For POTS, studies indicate autoimmune activity against G-protein coupled receptors (GPCRs), however, data is sparse. Exercise training is recommended in POTS, but little is known about clinical implementation.

Subjects: Patients in paper 1-4 (n=68-342) are from the Syncope Study of Unselected Population in Malmö (SYSTEMA), a cohort of patients evaluated for syncope and orthostatic intolerance at Skåne University Hospital (SUS), Malmö, Sweden. Paper 3 also includes patients (n=61) from the HeArt and bRain failure inVESTigation study (HARVEST) of admitted HF patients from SUS. Study 5 will include POTS-patients (n=200) from the Syncope Unit, SUS.

Methods and Results: Patients underwent active standing or head-up tilt test (HUT). Non-invasive cerebral oximetry measured SctO2 during HUT in paper 1, 3 and 4. GPCR activity in POTS versus controls and its association with symptoms were studied (paper 2). A cross-over study protocol of a 16-week exercise program was constructed. POTS ($p=0.023$) and HF patients ($p<0.001$) had lower SctO2 during orthostasis compared to SYSTEMA participants with normal HUT. Older patients with VVS and OH exhibited lower SctO2 prior to syncope than younger patients ($p<0.01$). Proteins activating adrenergic, muscarinic, and nociceptin receptors were highly predictive of POTS (Area-under-the-curve 0.88; 95% confidence interval 0.80-0.97).

Conclusion: Cerebral deoxygenation during orthostasis is notable in POTS, VVS, OH and HF, and may relate to aging in VVS and OH. The role of altered SctO2 in HF therapy and cognitive function should be further examined. High GPCR activity is predictive of POTS supporting autoimmune involvement. Exercise training in POTS warrants further studies for effective clinical implementation.

Key words: Autonomic nervous system, orthostatic intolerance, postural orthostatic tachycardia syndrome, vasovagal syncope, orthostatic hypotension, heart failure, exercise, cerebrovascular circulation, tilt-table test, G-protein-coupled receptors.

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To my family

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Preface

My journey into the field of the autonomic nervous system began during my first year of medical school. The delicate balance regulated by the autonomic nervous system, involving processes we often take for granted, like heart rate and blood pressure, fascinated me. When I learned about the research in Artur Fedorowski's lab on orthostatic intolerance syndromes and syncope, I knew it was something I wanted to explore further. The fact that cardiovascular dysautonomia hasn't been widely studied made it even more intriguing to me.

Cardiovascular autonomic dysfunction includes a range of disorders where the autonomic nervous system has trouble regulating heart and blood vessel functions. Conditions like postural orthostatic tachycardia syndrome, vasovagal syncope, and orthostatic hypotension can sometimes be hard to diagnose and seriously impact people's lives. Understanding these conditions and finding effective treatments is essential.

Joining Fedorowski's research group was a key moment in my academic journey. The group's welcoming and supportive atmosphere, along with the invaluable guidance of my supervisor Viktor Hamrefors, provided a great environment for developing my research skills. I'm grateful to have started my research career with such a dedicated team of researchers and colleagues who share my passion for understanding cardiovascular autonomic dysfunction.

This thesis aims to contribute to the growing knowledge of cardiovascular dysautonomia by trying to understand its causes and exploring potential diagnostic tools and treatments. While my findings may be a small piece of the larger puzzle, I hope they will add to the ongoing research in this important field.

Abstract

General Aim: To investigate novel diagnostic tools and treatment options for cardiovascular autonomic dysfunction (CVAD) patients, focusing on syncope, orthostatic intolerance, and heart failure (HF).

Background: CVAD includes common clinical entities such as vasovagal syncope (VVS) postural orthostatic tachycardia syndrome (POTS) and orthostatic hypotension (OH). Also, CVAD plays an important role in HF. Monitoring of cerebral tissue oxygenation (SctO2) during orthostasis may aid in understanding mechanisms in CVAD. For POTS, studies indicate autoimmune activity against G-protein coupled receptors (GPCRs), however, data is sparse. Exercise training is recommended in POTS, but little is known about clinical implementation.

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Populärvetenskaplig sammanfattning

Kardiovaskulär autonom dysfunktion innebär en störning i det autonoma nervsystemets styrning av hjärtat och blodkärlen. Detta kan leda till olika typer av svimningar, från godartade vasovagala svimningar (VVS) till livshotande arytmier. En annan form av autonom dysfunktion är ortostatisk intolerans, vilket innebär obehag eller svårighet att stå upp, så som ortostatisk hypotension (OH) och posturalt ortostatiskt takykardisyndrom (POTS). POTS drabbar oftast yngre personer och kännetecknas av en onormalt hög puls när man står upp och ortostatisk intolerans utan blodtrycksfall. Utöver detta uppvisar patienter med POTS ett flertal andra symtom från olika organsystem, så som kognitiva besvär, abnorm trötthet, bröstsmärtor och gastrointestinala besvär.

Trots att dessa tillstånd är vanliga i olika åldrar, är både diagnostik och behandling idag ofta otillräcklig. Handläggningen kompliceras dessutom av att de kliniska frågeställningarna spänner över flera olika discipliner inkluderande internmedicin, kardiologi och neurologi. Därtill verkar kardiovaskulär autonom dysfunktion vara en riskfaktor för hjärtkärlsjukdom i bredare bemärkelse, så som kranskärlssjukdom, hjärtsvikt och neurovaskulär sjukdom.

I detta avhandlingsprojekt studeras diagnostik och behandling av kardiovaskulär autonom dysfunktion, med fokus på svimning och ortostatisk intolerans, samt betydelsen av kardiovaskulär autonom dysfunktion vid hjärtsvikt.

Avhandlingen består av fem delprojekt:

1. Cerebral syresättning hos POTS patienter
Yrsel och kognitiva besvär är vanligt förekommande symptom vid POTS och man har tidigare haft teorier om att dessa symptom beror på en nedsatt blodtillförsel till hjärnan, trots ett normalt blodtryck. I delprojekt 1 studeras hjärnans syresättning hos POTS-patienter under tilt-test, där patienten tippas 60–70 grader uppåt för att framkalla symptom. POTS-patienter (n=34) och kontroller (n=34) genomgick tilt-testet med registrering av puls, blodtryck, EKG och cerebral syresättning. Resultaten visade att POTS-patienterna hade lägre syresättning i hjärnan trots bevarat blodtryck i stående. Detta samband var dock endast svagt kopplat till ökad hjärtfrekvens vid uppresning och hade inget samband med yrsel eller svimning under tilt-testet. Orsaken till den lägre syresättningen är fortfarande oklar och vi vet inte om det är en orsak eller en konsekvens av sjukdomen.

2. Autoimmunitet vid POTS
Det finns flera andra teorier om sjukdomsmekanismen vid POTS, däribland att det kan vara en autoimmun sjukdom, vilket stöds av att man i tidigare studier sett förekomst av autoimmuna antikroppar hos dessa patienter. I delprojekt 2 undersöktes aktiviteten i specifika receptorer hos POTS-patienter (n=47) och

friska kontroller (n=25) och om aktiviteten i receptorerna är associerade med POTS symptom framtagna från symptomskattningsformulär. I studien studerades 4 typer av receptorer (adrenerg receptor alpha1 och beta2, muskarin receptor typ2 (tre förstnämnda involverade vid reglering av hjärta och kärl) och opioid-receptor-like1 (involverad vid smärtperception)). Vi fann att blodprover från POTS patienterna aktiverade alla fyra receptorer i högre grad jämfört med blodprover från kontroller.

Adrenerg receptor alpha 1 aktivering var associerat med svårare symptom generellt men även för symptom i samband med längre tids stående och symptom vid gång. Alla 4 receptorer var associerade med synbesvär. Resultaten tyder på möjliga autoimmuna mekanismer vid POTS, men fler studier behövs för att vidare förstå detta möjliga samband.

3. Autonom Dysfunktion hos Hjärtsviktspatienter

Autonom dysfunktion spelar en viktig roll bakom sjukdomsmekanismen vid hjärtsvikt och tidigare forskning visar att hjärtsvikt kan ha skadlig påverkan på hjärnan. I delprojekt 3 studeras cerebral syresättning under tilt-test hos hjärtsviktspatienter (n=61) och kontroller med normal respons på ortostatisk provokation (n=60). Vi fann att hjärtsviktspatienter hade lägre syresättning i hjärnan både i liggande och efter tio minuters tilt test jämfört med kontrollpatienterna. Den lägre syresättningen i hjärnan var oberoende av skillnader i hjärtfrekvens och blodtryck. Konsekvenserna av en lägre SctO2 hos hjärtsviktspatienter på både kort och lång sikt bör studeras vidare.

4. Sambandet mellan åldrandet och syresättning i hjärnan hos patienter med vasovagal svimning och ortostatisk hypotension

Det är känt att benägenheten att svimma och utveckla blodtrycksfall är starkt beroende av åldern, men mekanismerna för dessa är inte helt kända. I delprojekt 4 studerades sambandet mellan ålder och syresättning i hjärnan under tilt-test hos patienter med VVS (n=139), OH (n=121) och kontrollpatienter med normalt tilt-test (n=82). Resultaten visade bland annat att syresättningen i hjärnan 30 sekunder innan svimning, sjönk med åldern, bland patienter med VVS och OH, oberoende av den aktuella blodtrycksnivån. Det talar för att andra mekanismer än konventionella mätningar av puls och blodtryck är av betydelse vad gäller hjärnans syretillförsel och benägenhet att utveckla svimning i olika åldrar.

5. Träningsprogram för POTS

Enligt en internationell konsensus rekommenderar experterna fysisk aktivitet och träning som kompletterande behandling vid POTS. Ett anpassat träningsprogram med fysioterapeuter utgör redan idag en del av den kliniska behandlingen men problemet är att detta är resurskrävande och gör att inte alla patienter med behov får ta del av optimal träning anpassad för POTS. Ett strukturerat träningsprogram som genomförs i grupp skulle eventuellt kunna öka möjligheten för fler patienter att ta del av träningsprogram anpassat för POTS. Delarbete 5 består av ett

studieprotokoll för en cross-over studie med 200 POTS patienter som randomiseras till 2 olika grupper. Syftet med cross-over-studien är att utvärdera om ett träningsprogram i grupp lett av fysioterapeuter med speciellt intresse för POTS, som redan görs som del av sjukvården, under fyra månader har positiv effekt på symptom och hemodynamik vid POTS.

Sammanfattningsvis bidrar dessa studier till ökad förståelse för möjliga sjukdomsmekanismer, diagnostiska metoder och behandlingar, vilket på sikt kanske kan förbättra livskvaliteten för drabbade individer. Fortsatt forskning är avgörande för att utveckla mer effektiva diagnostik- och behandlingsmetoder.

List of papers

This thesis is based on the following papers:

I. **Kharraziha I**, Holm H, Bachus E, Melander O, Sutton R, Fedorowski A, Hamrefors V. Monitoring of cerebral oximetry in patients with postural orthostatic tachycardia syndrome. Europace. 2019 Oct 1;21(10):1575-1583. doi: 10.1093/europace/euz204.

II. **Kharraziha I**, Axelsson J, Ricci F, Di Martino G, Persson M, Sutton R, Fedorowski A, Hamrefors V. Serum Activity Against G Protein-Coupled Receptors and Severity of Orthostatic Symptoms in Postural Orthostatic Tachycardia Syndrome. Journal of American Heart Association. 2020 Aug 4;9(15): e015989. doi: 10.1161/JAHA.120.015989. Epub 2020 Jul 30.

III. **Kharraziha I**, Holm H*, Magnusson M, Wollmer P, Molvin J, Jujic A, Fedorowski A, Bachus E, Hamrefors V**. Impaired cerebral oxygenation in heart failure patients at rest and during head-up tilt testing. ESC Heart Failure. 2021 Feb;8(1):586-594. doi: 10.1002/ehf2.13128. Epub 2020 Dec 9.

IV. **Kharraziha I**, Torabi P, Johansson M, Sutton R, Fedorowski A, Hamrefors V. The Influence of Age on Cerebral Tissue Oxygenation in Vasovagal Syncope and Orthostatic Hypotension. Journal of Clinical Medicine. 2022 Jul 25;11(15):4302. doi: 10.3390/jcm11154302.

V. **Kharraziha, I**., Zulj, R., Holm Isholth, H. Fedorowski, A. Hamrefors, V. Evaluation of a structured exercise program in clinical practice for postural orthostatic tachycardia syndrome patients – a protocol for a randomized cross-over study. 2024. (Manuscript)

* Kharraziha I and Holm H shared first authorship.
** Bachus E and Hamrefors V shared senior authorship.

Author's contributions to the papers

Paper I-V

Conceptualisation: The author was involved and shaped the foundational ideas in paper 2-5. In paper 5, the author was responsible for the design of the cross-over study, with some input from supervisors. In paper 1, the author was not involved in the design of the study.

Pre-processing: Collection of hemodynamic parameters and cerebral oximetry in paper 1 and 4. Collection of symptom questionnaires from patients in paper 2.

Post-processing: Refined and organized the collected data.

Statistical analysis: Performed all the statistical analyses in papers 1, 3 and 4. In paper 2, the author performed all the statistical analysis except for the ROC analysis. In paper 5, the author performed power calculation.

Interpretation: Contributed significantly to the interpretation of the results.

Writing/publishing: Throughout the projects, the author maintained active collaboration with fellow researchers, was the corresponding author for paper 2 and 4, and drafted all manuscripts.

Abbreviations

ADRA1	Adrenergic α1 receptor
ADRB2	Adrenergic β2 receptor
AUC	Area under the curve
CBF	Cerebral blood flow
CHRM2	Cholinergic muscarinic type 2 receptor
CO	Cardiac output
CO2	Carbon dioxide
CVAD	Cardiovascular autonomic dysfunction
DBP	Diastolic blood pressure
FRET	Fluorescence resonance energy transfer
GPCR	G-protein coupled receptors
HARVEST	HeArt and bRain failure inVESTigation study
HF	Heart failure
HR	Heart rate
HUT	Head-up tilt test
IVIG	Intravenous immunoglobulin
NIRS	Near infrared spectroscopy
NTG	Nitroglycerin
NYHA	New York Heart Association
OH	Orthostatic hypotension
OHDAS	Orthostatic Hypotension Daily Activity Scale
OHSA	Orthostatic Hypotension Symptom Assessment
OHQ	Orthostatic Hypotension Questionnaire
OPRL1	Opioid receptor-like 1
POTS	Postural orthostatic tachycardia syndrome
ROC	Receiver operating characteristic
SBP	Systolic blood pressure
SctO2	Cerebral tissue oxygenation
SF-36	36-Item Short Form Health Survey
SYSTEMA	Syncope study of unselected population in Malmö
VVS	Vasovagal syncope

Introduction

Autonomic Nervous System

The autonomic nervous system (ANS) controls involuntary functions and influences the activity of internal organs such as blood vessels, the heart, lungs, gastrointestinal tract, kidneys, pupils, sweat, salivary, and digestive glands (1, 2). The ANS is often divided into three divisions: the sympathetic nervous system (SNS), parasympathetic nervous system (PNS) and enteric nervous system (ENS) (1). The ENS, primarily focused on reflex pathways that control digestive functions (3), is often considered separately due to its localization within the gastrointestinal organs (4). The SNS prepares the body for "fight or flight" situations by increasing heart rate (HR), blood pressure, and blood flow to skeletal muscles, while decreasing gastrointestinal peristalsis. Conversely, the PNS supports "rest and digest" processes by reducing HR and blood pressure and enhancing gastrointestinal peristalsis and digestion (3, 4).

The SNS originates from the thoracic and lumbar spinal cord whereas the PNS emerges via cranial nerves and sacral spinal cord (5). The SNS and PNS consist of preganglionic neurons with a cell body in the central nervous system and a postganglionic neuron with a cell body in the periphery innervating target cells (1) (see figure 1 for a schematic overview). The presynaptic neurons of both the SNS and PNS use acetylcholine as neurotransmitter. In postsynaptic neurons, norepinephrine is the main neurotransmitter in the SNS, whereas acetylcholine is used in postsynaptic parasympathetic neurons (1). These neurotransmitters bind to specific G-protein coupled receptors (GPCR) causing conformational changes, which in turn causes a cycle of G-protein activation and inactivation. This process allows the G-protein to modulate enzyme and ion channel activities, regulating the formation and concentration of intracellular signaling molecules that carry messages from outside the cell to inside, consequently leading to different cellular responses (6). Please see figure 2, showing a schematic overview of neurotransmitter activation of GPCR.

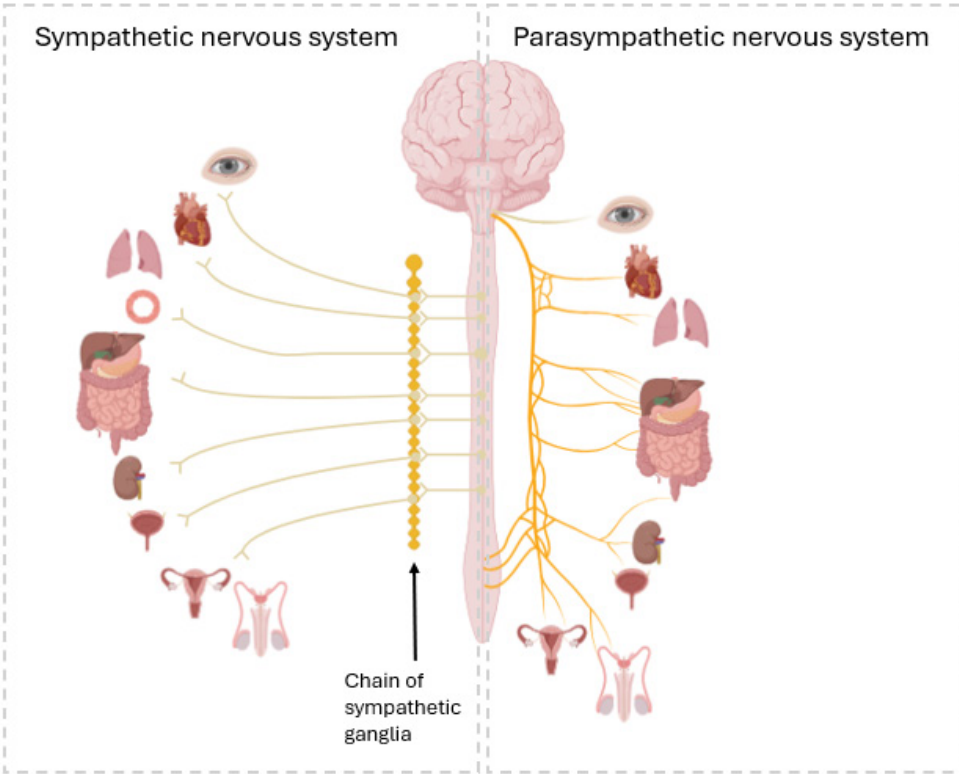


Figure 1. Anatomical overview of the autonomic nervous system. The sympathetic nervous system (SNS) originates from the thoracic and lumbar spinal cord whereas the parasympathetic nervous system (PNS) emerges via cranial nerves and sacral spinal cord. The SNS and PNS consist of preganglionic neurons with a cell body in the central nervous system and a postganglionic neuron with a cell body in the periphery innervating target cells. Created in BioRender.com.

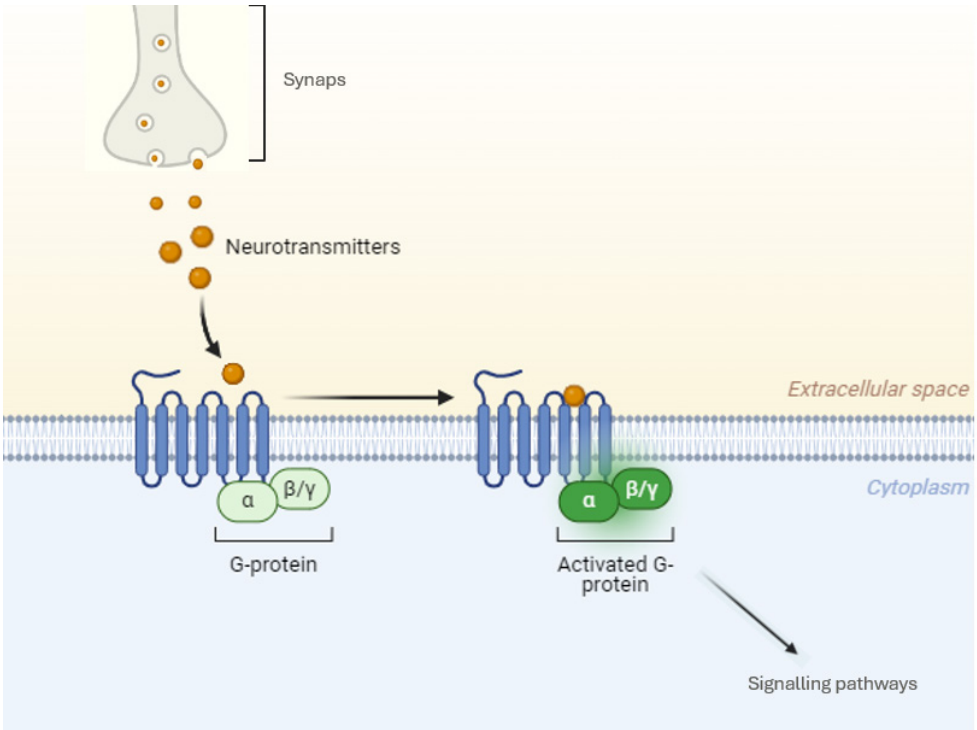


Figure 2. G-protein coupled receptor (GPCR). Neurotransmitters causes a conformational change to the GPCR, triggering a cycle of G-protein activation and inactivation. This process allows the G-protein to modulate enzyme and ion channel activities, regulating the formation and concentration of intracellular signaling molecules that carry messages from outside the cell to inside, triggering specific cellular responses. Adapted from " G-Protein-Coupled Receptors", by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates.'

Clinical Manifestations of Autonomic Dysfunction

The cardiovascular branch of the ANS is responsible for regulation of HR, and blood pressure, and to maintain homeostasis during physiological stresses such as standing up or during exercise (1).

Standing up puts the brain in a notably disadvantageous position (7). When standing, blood shifts from the chest to the lower abdomen and legs, and from the vasculature into the interstitial space. In a healthy person, standing reduces venous return to the heart which in turn reduces cardiac output (CO). When the systemic pressure decreases, compensatory sympathetic activation increases HR and vascular tone via the baroreceptor reflex (8), which eventually will lead to restored venous return and CO. Cerebral blood flow (CBF) is dependent on cerebral autoregulation, which strives to keep CBF constant within a mean arterial pressure range of 50–150 mmHg (9). When the ANS fails to adjust to

orthostatic changes, symptoms of cerebral hypoperfusion such as dizziness, cognitive deficits, and loss of consciousness can occur (10). Failure of the ANS to maintain homeostasis under such, and other, conditions is referred to as cardiovascular autonomic dysfunction (CVAD) or dysautonomia (11).

Postural Orthostatic Tachycardia Syndrome

POTS is a disease of unknown origin characterized by symptoms of orthostatic intolerance and a HR increase of >30 beats per minute when upright without OH (12, 13). In addition to orthostatic intolerance, patients with POTS may experience debilitating symptoms only partly related or unrelated to orthostasis such as dizziness, fatigue, "brain fog", chest pain, gastrointestinal problems etc. The disease mostly affects younger individuals, and most are female (≈80%) (12). The prevalence of POTS has been suggested to be approximately 0.2 % (13). However, the prevalence is difficult to estimate since it is likely that many POTS patients are un- or misdiagnosed due to lack of knowledge among physicians (14).

Even though the pathogenesis is still unclear, several potential underlying mechanisms in POTS have been suggested, such as autonomic denervation, hyperadrenergic stimulation, hypovolemia, and autoantibodies against adrenergic receptors (12).

Light headedness and neurocognitive deficits are some of the most disabling symptoms experienced by POTS patients and it has been hypothesized that these symptoms are dure to cerebral hypoperfusion, despite preserved systemic blood pressure (15). Previous research have studied cerebral circulation among POTS patients during HUT or active standing but results have been inconsistent (16-20). Thus, we aimed to study differences in cerebral tissue oxygenation (SctO2) during head-up tilt test (HUT) among POTS patients and controls in relation to hemodynamic factors and symptoms (paper 1).

As previously mentioned, it has been hypothesized that POTS is an autoimmune disease, which is supported by several previous findings (12). First, the predominance of women and the onset of POTS following a viral infection, vaccination, or trauma share similarities with the characteristic traits of autoimmune diseases (21). Second, it has been found that nearly one fourth of POTS patients have positive antinuclear antibodies and a higher prevalence of other autoimmune disease such as Hashimoto's Disease, Rheumatoid Arthritis and Sjögrens Syndrome (22). Third, previous studies have found presence of antibodies against adrenergic and cholinergic GPCRs in POTS (23-25). To learn more about the role of GPCRs in POTS, we have studied serum activity against GPCRs in relation to symptoms in POTS patients and controls (paper 2).

Another important clue behind the pathophysiology of POTS has been suggested to be cardiovascular deconditioning and reduced standing stroke volume (26-28).

This concept is supported by previous research that found that physical reconditioning with short-term exercise training significantly increased peak oxygen uptake (an indicator of physical fitness), increased heart size, expanded blood and plasma volume, as well as improved POTS symptoms and quality of life (27-30). Furthermore, 50–70% of POTS patients who finished the 3-month program of endurance training no longer meat the hemodynamic criteria for POTS (27, 31, 32). Exercise training should be considered as a complementary therapy in POTS according to international guidelines (13). However, there is limited knowledge of how to effectively incorporate exercise training into clinical practice for patients with POTS. Therefore, we aimed to evaluate the impact of a 16-week tailored group exercise training program on individuals with POTS (paper 5).

POTS is increasingly recognized affecting young individuals, often causing severely disabling symptoms that not only impact these individuals but also result in significant costs for society (33). In fact, a previous study on 5,556 adult POTS patients found that 50.2 % were unemployed, and a total of 74% had been unable to work for a period of greater than one week due to their POTS symptoms (33). This empathizes the importance of learning more about POTS and finding an effective treatment for these patients.

Syncope, orthostatic hypotension, and aging

Syncope may reduce quality of life (34) and accounts for a substantial number of emergency admissions in both younger and older persons (35). Syncope is common, affecting approximately 50% of individuals by the age of 80 (36). In addition, syncope is responsible for 1–3% of all hospital admissions and emergency room visits (36). In the older person in particular, syncope carries a high morbidity and mortality (37).

Vasovagal syncope is the most common form of syncope (38). A vasovagal reflex is a sudden autonomic activation (in response to a trigger such as emotional stress or standing) leading to a vagal chronotropic and dromotropic inhibition and a peripheral vasodilation. This effect leads to bradycardia or asystole and hypotension, in turn leading to cerebral hypoperfusion and syncope. The pathophysiology is unclear but involves a complex interplay between the ANS and cardiovascular responses (39).

In contrast to VVS, OH is characterized by a progressive and sustained fall in blood pressure upon standing. OH, may lead to adverse events, such as traumatic injuries and syncope and may negatively affect quality of life (8). According to international guidelines (35) OH is defined as a decrease in systolic blood pressure (SBP) from baseline value >_20 mmHg or diastolic BP >_10 mmHg, or a decrease in SBP to < 90 mmHg. Three major subtypes of OH have been described: initial

orthostatic hypotension, classic orthostatic hypotension, and delayed orthostatic hypotension (8).

With aging, alterations occur in the cardiovascular system, including impaired regulation of HR, blood pressure, and CBF control (40, 41). Multimorbidity and polypharmacy in combination with these cardiovascular changes may explain why older patients are more susceptible to syncope and OH (35). First syncope incidence has a bimodal distribution with the highest incidence among individuals below 30 and above 60 years of age (42). We hypothesized that an impairment of the cerebral autoregulation increases with age which may potentially contribute to these differences, especially the increased incidence of syncope after 60 years (43). However, previous studies have investigated age-related differences on cerebral circulation during orthostatic provocation in healthy individuals, but the results have been inconsistent (44-47). In project 4, we aimed to investigate the association between SctO2 and age during HUT in VVS, OH patients and patients with normal response to HUT, to learn more about these age-related differences in syncope and orthostatic intolerance patients.

Autonomic Dysfunction and Heart Failure

Heart failure (HF) is a clinical syndrome marked by reduced CO and/or increased intracardiac pressures, leading to symptoms such as fatigue, dyspnea, and peripheral and/or pulmonary congestion (48).

In response to a decrease in CO and tissue hypoperfusion in HF patients there is often a sustained increase in sympathetic and neurohormonal activity (49). The Renin-Angiotensin-Aldosterone System contributes to HF by promoting vasoconstriction, sodium and water retention, and myocardial remodelling, which exacerbates cardiac dysfunction and progression of the disease (50). An aspect of HF that has not been extensively studied is its potential adverse effects on the brain (51) and autonomic nervous system (52), possibly due to cerebral hypoperfusion resulting from systemic hypotension . This could partially explain why cognitive dysfunction is more prevalent in HF patients and why these patients often experience a faster decline in cognitive function compared to individuals without HF (53).

As mentioned earlier, in healthy individuals, standing induces venous pooling to the lower limbs and splanchnic region, reducing venous return and CO. Consequently, autonomic responses are triggered, which, if inadequate or impaired, may lead to reduced CBF (7, 8). In HF, where CO is already compromised, orthostatic provocation may increase the risk of cerebral hypoperfusion, further predisposing individuals to chronic cerebral ischemia and cognitive impairment (54). Previous studies have shown that HF patients demonstrate abnormal hemodynamic responses to standing (54-56) and experience

a greater decline in CBF (55, 57) during orthostatic provocation compared to healthy individuals. However, changes in SctO2 during orthostatic stress in HF patients remain underexplored. Thus, in project 3, changes in SctO2 during orthostatic provocation among HF patients was studied.

Cerebral oximetry

Cerebral oximetry, was used to study SctO2 in paper 1, 3 and 4. Cerebral oximetry, measured with near-infrared spectroscopy (NIRS) has long been used in the field of anaesthesia, primarily for monitoring cerebral oxygenation and ensuring adequate brain perfusion during surgical procedures (58). Its ability to provide real-time, non-invasive measurements of SctO2 has made it a valuable tool in the anaesthetic management of patients (59).

In recent years, NIRS has gained increased attention for its application in the assessment of patients with syncope and orthostatic intolerance. Researchers and clinicians have begun to explore the use of NIRS in these conditions to better understand the underlying mechanisms of cerebral hypoperfusion in these patients (60).

NIRS measures the ratio of oxygenated hemoglobin (Hb) to total Hb, which reflects a proportional mix of arterial and venous blood in the outer regions of the frontal hemispheres (60). Near-infrared light, ranging from 700 to 1,000 nm, passes through tissues like skin and bone with minimal absorption, while Hb exhibits a well-defined absorption spectrum that changes with oxygen binding. Because oxygenated Hb and deoxygenated Hb have different absorption spectra, their proportion can be calculated (59). In healthy individuals, normal SctO2 levels have been established to range between 60% and 80% (59). A figure of the NIRS probe is shown below (Figure 3).

The primary advantage of NIRS is its ease of use. Unlike transcranial doppler derived CBF measurements, NIRS requires no specialized training (61). However, NIRS does not measure CBF directly unless a flow tracer, such as a short breath of 100% oxygen or an injection of a contrast agent, is used (62). However, a previous study found that changes in SctO2 measured by NIRS were correlated with changes in CBF derived from transcranial doppler in covid-19 patients admitted to the intensive care unit (63), supporting the idea that NIRS may serve as a surrogate for CBF measurements.

By applying cerebral oximetry during orthostatic provocation through HUT, it has been observed that SctO2 decreases slightly even in normal subjects, although the decrease is small (64-66). In contrast, patients with syncope and orthostatic intolerance exhibited a more pronounced decrease in cerebral tissue oxygenation during HUT (64-66).

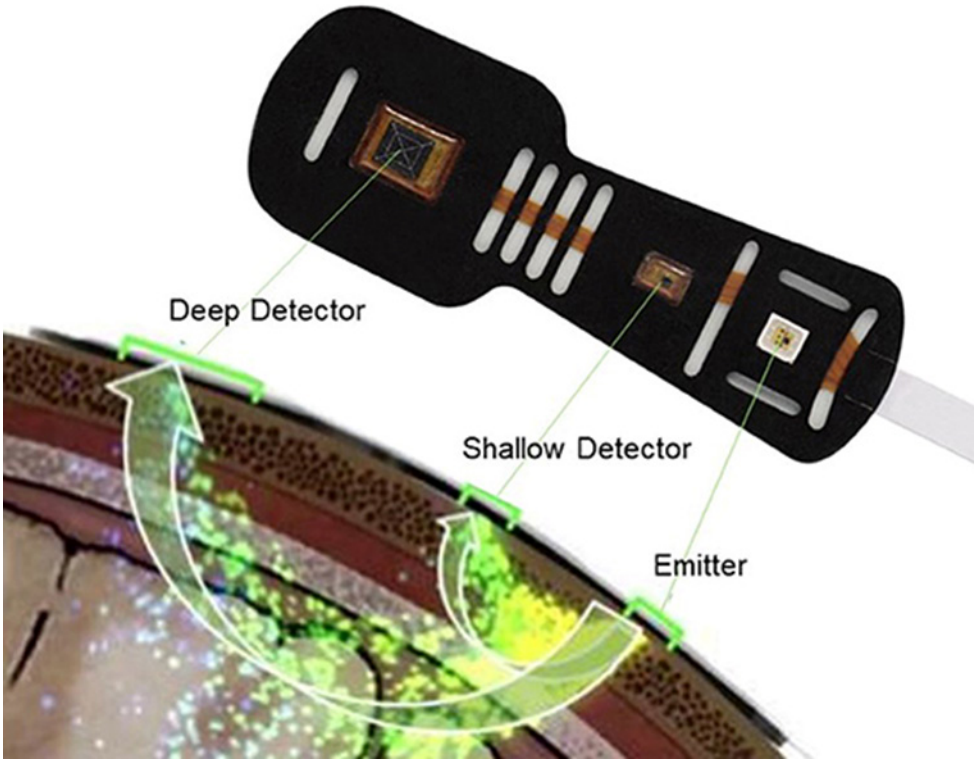


Figure 3. Measurement of cerebral tissue oxygenation using near-infrared spectroscopy (NIRS). The NIRS probe, attached to the forehead, emits near-infrared light, which passes through tissues such as skin and bone with minimal absorption. Hemoglobin (Hb), however, has a well-defined absorption spectrum that varies depending on its oxygenation state. Light attenuation is detected by both deep and shallow detectors, as illustrated in this figure. Since oxygenated and deoxygenated Hb have different absorption spectra, their proportions can be calculated. Reprinted from: I. Kharraziha et al. "Cerebral Oximetry in Syncope and Syndromes of Orthostatic Intolerance." Frontiers in cardiovascular medicine vol. 6 171. 22 Nov. 2019, doi:10.3389/fcvm.2019.00171. Licensed under CC BY.

The use of cerebral oximetry in patients with syncope has led to several significant findings. One important discovery is the ability to predict the onset of VVS before noticeable changes in hemodynamic parameters occur, by showing a gradual decrease in SctO2 prior to syncope (65, 66).

A study by Bachus et al. (65) reported a significant decrease in SctO2 one minute before reflex activation, whereas mean arterial pressure (MAP) did not show a significant decrease at this time. Another notable finding from the same study was that syncope occurred when SctO2 fell below 60%. The decrease in SctO2 helps explain the discrepancy between symptoms reported by patients and the hemodynamic parameters observed, particularly during the prodromal phase of VVS or unexplained symptoms of orthostatic intolerance.

Furthermore, the addition of cerebral oximetry to HUT allows a more sensitive detection of orthostatic intolerance and may reflect disrupted homeostasis of cerebral oxygenation in POTS (60, 64).

Lastly, NIRS has previously been studied in HF patients, showing reduced frontal brain activity as assessed by NIRS. The reduction was associated with high levels of anxiety and decreased cognitive function (67). With its increasing use in research, cerebral oximetry has the potential to reveal factors contributing to cerebral hypoperfusion and uncover previously unknown mechanisms underlying syndromes associated with recurrent syncope and orthostatic intolerance.

Aims

Even though cardiovascular dysautonomia affects many individuals, current diagnostic and treatment approaches remain insufficient. Hence, this PhD project aims to study novel diagnostic methods and management tools for various conditions associated with CVAD, focusing particularly on syncope and common syndromes of orthostatic intolerance (POTS and OH). It also explores the role of CVAD in HF patients.

Project specific research questions:

1. What significance does SctO2 have in POTS patients and what is the relationship with hemodynamic factors and symptoms? (Paper I)
2. What role does autoimmune antibodies against G-protein coupled receptors have in POTS and what is the diagnostic value of these receptors? (Paper II)
3. What significance does SctO2 have in HF patients and what is the relationship with hemodynamic factors? (Paper III)
4. Are there age-related differences in SctO2 during head up tilt test in reflex syncope and OH patients? (Paper IV)
5. What is the effect of a 16-week training program held in groups lead by physiotherapists with special interest in POTS, on symptoms, maximal workload and hemodynamics in POTS? (Paper V)

Materials and Methods

Cohorts

Syncope Study of Unselected Population Malmö (SYSTEMA)

Paper 1, 2, 3 and 4 were based on the Syncope Study of Unselected Population Malmö (SYSTEMA) cohort. The SYSTEMA cohort consists of patients with unclear syncope and orthostatic intolerance who were evaluated at a tertiary centre at Skåne University Hospital, Malmö, Sweden between 2008 and 2021. Patients were referred from other clinics and primary care centres mainly in Skåne but also from other parts of Sweden. The characteristics and diagnoses in 2663 subjects from SYSTEMA who have been analysed in detail were recently published (68).

Patients with confirmed cardiac, metabolic, neurological, toxic, and other aetiologies of transient loss of consciousness prior to HUT, as well as patients with advanced dementia or physical disability were not included in SYSTEMA. All patients underwent cardiovascular autonomic testing, including HUT and additional tests, such as ambulatory ECG or 24-hour blood pressure monitoring when clinically indicated. Since 2013, cerebral oximetry has been routinely performed during HUT.

In paper 2, the control group consisted of healthy volunteers, and were not recruited from the SYSTEMA cohort (see details under project specific methods).

HeARt and brain failure inVESTigation study (HARVEST)

Paper 3 was based on both the SYSTEMA cohort as well as the Swedish Heart and Brain Failure Investigation Study (HARVEST-Malmö). HARVEST is an ongoing (2014 –), prospective study, on HF patients (newly diagnosed or exacerbated chronic heart failure), admitted to the cardiology or internal medicine wards at Skåne University Hospital in Malmö, Sweden (69). At the time of paper 3, a total of 316 patients had been included to the cohort. In paper 3, patients with NYHA class IV at the time of enrolment were excluded. Patients with NYHA class I-III, enrolled in HARVEST-Malmö, where invited to undergo HUT once their HF was stabilized.

Ethical Considerations

All research projects described in this thesis received ethical approval (Paper 1: DNR 08/82, 2015/224, and 2017/295; Paper 2: DNR 08/82 and 17/295; Paper 3: DNR 08/82 and 2013/360; Paper 4: DNR 08/82, DNR 2015/224; Paper 5: 2022-03186-01). Written informed consent was obtained from all participants (Papers 1-4).

CVAD, the focus of this research, significantly impacts patient well-being, necessitating ongoing research into its pathophysiological mechanisms and potential treatments. Ethical research practices, especially in clinical studies, require careful consideration of several factors:

Voluntary Participation: Participation must be voluntary, with patients free to withdraw at any time without affecting their medical care. This is critical given the potential dependency of patients on physicians involved in the research.

Informed Consent: Detailed consent forms were provided. Participants were reassured about the voluntary nature of their involvement and their right to discontinue participation without consequence.

Confidentiality and Data Protection: Stringent measures were adopted to ensure the privacy and confidentiality of participant data. All sensitive information was password-protected and accessible only to the research team. The blood samples (paper 2) were marked with a serial number and could only be traced to the patient by primary investigators in our research group via a secure database.

Clinical Procedures and Potential Discomfort: Various procedures were necessary for the studies. The HUT tests (Papers 1, 3, and 4), although causing discomfort like dizziness or syncope in some cases, were conducted with precautions to minimize risk, especially excluding the most vulnerable patients such as those with heart failure, NYHA class IV. Also, HF patients did not receive sublingual NTG. If patients experienced symptoms indicating presyncope or signs of clinical instability, the patient was immediately tilted back to supine. Cerebral oximetry, performed in Papers 1, 3, and 4, is a non-invasive method that poses minimal risk and discomfort. In paper 2, blood tests to analyse GPCR activity were performed. Blood testing may cause discomfort for patients, but it does not pose significant health risks. In paper 5, a 16-week exercise training program for POTS patients was designed with the understanding that while exercise is beneficial, its implementation in clinical practice requires careful study. Despite the physical challenge, the potential therapeutic benefits were deemed to outweigh the risks.

In summary, while ethical challenges such as potential pressure to participate in a study or data mishandling exist, careful planning and adherence to ethical guidelines aimed to ensure that the benefits of gaining knowledge significantly

outweighed any risks. Each study was designed with a strong ethical framework to protect participants and ensure the integrity of the research process.

Head-up Tilt Test

Head-up tilt test (HUT) is a recognized method that has been used for more than half a century and is an important diagnostic tool within syncope and orthostatic intolerance. It allows for the observation of hemodynamic changes during orthostatic provocation (70). HUT was performed on all patients in papers 1, 3 and 4 according to the Italian protocol, as previously described (65). In short, patients were tilted head-up to 60-70 degrees after a supine rest of 15 minutes. If syncope or pre-syncope with typical prodromal symptoms occurred, the test was considered positive, and the patient was immediately tilted back to supine. Blood pressure was continuously measured using a photoplethysmographic device (Nexfin, BMEYE, Amsterdam, The Netherlands or Finapres Nova, Finapres Medical Systems, PH Enschede, The Netherlands), together with peripheral oxygen saturation (SPO2) and electrocardiogram. Sublingual nitroglycerin (NTG) was administered after 20 minutes of tilt in patients with history of syncope if the unmedicated phase was negative. Consequently, in paper 1 and 3, only the first 20 min of measurements were included as administration of NTG was incompatible with study aims. Patients with HF in paper 3, did not receive NTG. Patients with suspect POTS were asked to abstain from their regular medications 48 hours before examination in order to confirm or exclude POTS diagnosis and analyse untreated haemodynamic responses. All other patients were asked to continue with their medications as normal before the HUT. This is especially relevant for syncope patients, for whom the real-life scenario (i.e. regular drug intake) was recreated as far as possible,

Cerebral Oximetry

Cerebral oximetry, measured by NIRS, was performed in paper 1, 3, and 4 using the Fore-Sight absolute cerebral oximeter (CAS Medical Systems Inc., Branford, CT, USA), as previously described in a SYSTEMA cohort study (65). The Fore-Sight monitor has two sensors for bilateral monitoring and projects four specific wavelengths (690, 780, 805, and 850 nm) into the brain. Absolute cerebral oximetry and hemodynamic parameters were measured simultaneously, in the same file time synchronized. Please see figure 4 demonstrating the HUT test and cerebral oximetry monitoring.

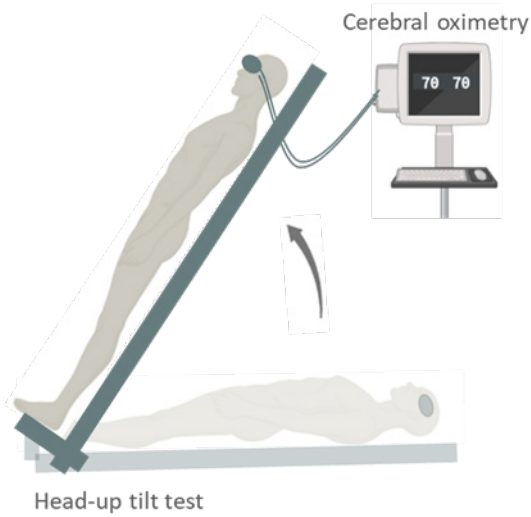


Figure 4. Schematic figure of the head up tilt test and cerebral oximetry. Patients were tilted to a head-up angle of 60-70 degrees after a supine rest of 15 minutes. If syncope or pre-syncope with typical prodromal symptoms occurred, the test was considered positive, and the patient was immediately tilted back to supine. Blood pressure, peripheral oxygen saturation, electrocardiogram and cerebral tissue oxygenation were continuously monitored during the head-up tilt test. Created in BioRender.com.

Symptom Questionnaires

Orthostatic Hypotension Questionnaire

The Orthostatic Hypotension Questionnaire (OHQ) is a symptom questionnaire that has previously been validated for OH patients (71) but has also been used for evaluating POTS related symptoms (12, 72, 73). As previously described (71), the OHQ is divided into two categories: Orthostatic Hypotension Symptom Assessment (OHSA) and Orthostatic Hypotension Daily Activity Scale (OHDAS). OHSA consists of 6 questions regarding the following symptoms: dizziness, light-headedness, feeling faint, or feeling like you might blackout: problems with vision (eg, blurring, seeing spots, and tunnel vision); generalized weakness; fatigue; trouble concentrating; and head/neck discomfort. The OHDAS includes 4 questions and assesses how patients' symptoms affect daily activities (standing for long and short duration, walking for short or long duration). Patients are asked to answer the symptom questionnaire with a recall period over the past week. All questions are scored on a scale from 0 to 10, with 0 indicating no symptoms and 10 indicating worst possible symptoms. The composite OHQ score is calculated by averaging the OHSAS and the OHDAS. Answers that are marked with a zero or "cannot be done for other reasons" at baseline are not included in the calculation.

Malmö POTS symptom score

Malmö POTS Symptom Score (MAPS) is a symptom scoring system that uses a visual analogue scale from 0 (no symptoms) to 10 (very pronounced symptoms) for POTS patients to self-assess their symptom severity over the past 7 days. The questionnaire focuses on the patient's perception of 12 common symptoms. It includes five cardiac symptoms (palpitations, dizziness, presyncope, dyspnea, and chest pain) and seven non-cardiac symptoms (gastrointestinal problems, insomnia, concentration problems, headache, myalgia, nausea, and fatigue). The total score can range from 0 to 120 points. Further details about MAPS are available elsewhere (74).

SF-36 Health questionnaire

The 36-Item Short Form Health Survey (SF-36) is a 36-item, patient-reported questionnaire, that evaluates eight health domains: physical functioning, bodily pain, role limitations due to physical health, role limitations due to emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. Each domain is scored on a scale from 0 to 100, where higher scores indicate a better health state. More details on the SF-36 Health Questionnaire have been described previously (75).

Project specific methods and statistical analysis

Data were analysed using SPSS software version 25 (SPSS, Chicago, IL, USA). A P-value <0.05 was considered significant for all tests. P values are presented unadjusted for multiple testing. However, when interpreting the results, we accounted for multiple testing. In papers 1, 2, 3, and 4 quantitative variables were assessed as normally distributed after visual inspection of distribution plots. See below for project specific details on statistical analysis. A flow chart on selection of study participants in papers 1-5 is presented in figure 5.

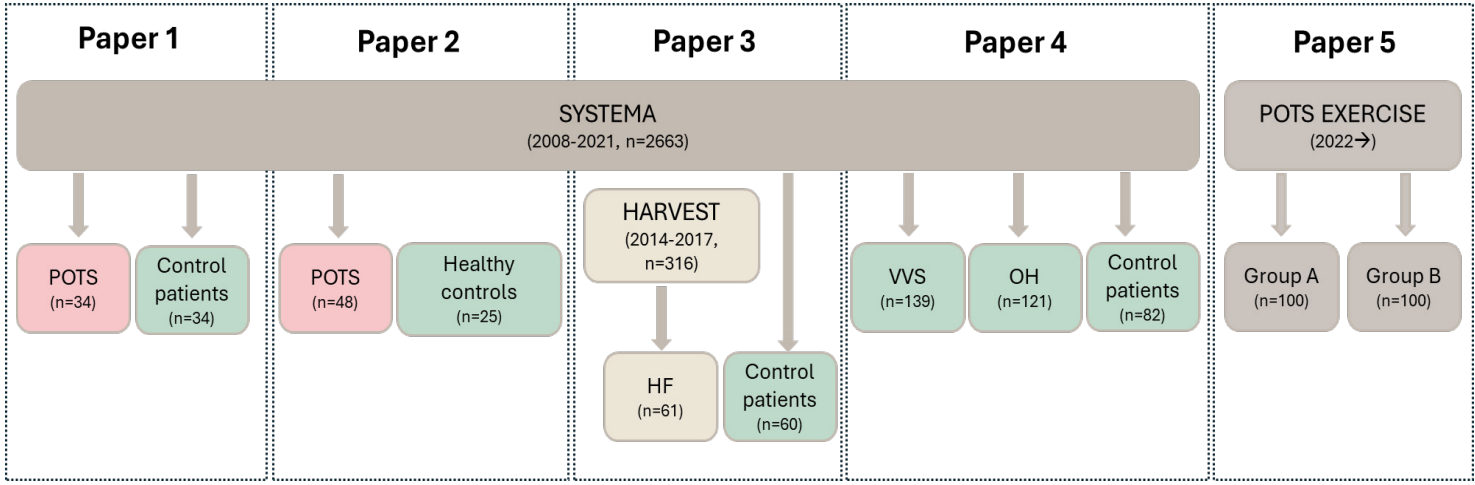


Figure 5. Flow chart on selection of study participants (papers 1-5). Paper 1 included 34 POTS patients and 34 age and sex matched controls from the SYSTEMA cohort between 2013-2018. In paper 2, 48 POTS patients were included from the SYSTEMA cohort, whereas the controls consisted of 25 healthy volunteers recruited through personal invitation, included between 2017-2018. Paper 3 was based on 61 heart failure patients from the HARVEST cohort and 60 control patients from the SYSTEMA cohort, included between 2014-2017. Paper 4 was based on the SYSTEMA cohort (2016-2020), where a total of 139 VVS, 121 OH and 82 control patients were included. Lastly, a cross-over study of a 16-week exercise training program will be conducted from 2022, including 200 POTS patients in total. Abbreviations: POTS, postural orthostatic tachycardia syndrome; HF, heart failure; VVS, vasovagal syncope; OH, orthostatic hypotension; SYSTEMA, syncope study of unselected population in Malmö; HARVEST, HeARt and brain failure inVESTigation study.

Paper 1

A total of 34 patients with POTS and 34 one-to-one age and sex matched controls with normal response to HUT, excluding NTG provocation, were included from the SYSTEMA cohort between 2013-2018, which consisted of 2074 patients at the time of the study. Cerebral oximetry had been performed on 354 patients, and 34 of these patients were diagnosed with POTS according to the Heart Rhythm Society criteria (13). Additionally, a senior cardiologist who specializes in POTS reviewed and confirmed each diagnosis. Patients with spontaneous VVS or OH during HUT, coronary artery disease, diabetes mellitus, or stroke were excluded from controls. HUT tests were performed as described above. ScO2 values from cerebral oximetry were collected in supine position and at 1, 3, and 10 minutes of HUT. Minimum SctO2 during the test (SctO2 min) was defined as the minimum SctO2 value at any time prior to reflex activation. SctO2 delta was defined as the difference in SctO2 in supine position and minimum, 1, 3, and 10 minutes of HUT, respectively. In this study, only the first 20 minutes of measurements were included, as afterwards sublingual NTG was routinely administrated and was therefore considered incompatible with the study aims.

Statistical analysis paper 1

Mean SctO2 at rest and during orthostatic provocation were compared between POTS and control patients with independent samples t-test. The proportion of patients with SctO2 < 65 % (lower limit of normal in subjects with normal HUT (65), was compared between POTS and control patients, using Pearsons $\chi 2$ test. The relationship between SctO2, SBP, and HR at the defined time points, during HUT was linearly assessed, after adjusting for age. Also, the relationship between SctO2 values and reported dizziness and syncope during HUT were studied, as well as differences in SctO2 according to sex, using independent samples t-test.

Paper 2

A total of 48 POTS patients from the SYSTEMA cohort were included. A control group of 25 healthy volunteers were recruited through personal invitation (e.g., Skåne University Hospital staff, medical students, and younger participants from population-based epidemiologic programs in Malmö, Sweden). Patient and control recruitment was conducted between 2017-2018. The POTS patients had a confirmed diagnosis by one of our cardiologists with special expertise in POTS. Also, all POTS patients had a previous positive HUT which qualified them as POTS in this study. Controls did not perform HUT. Blood samples were collected from all patients and controls (n=73) and sent for analysis at the Center for Apherensis and Stem Cell Handling at Karolinska University Hospital in Stockholm, Sweden.

Out of the 73 subjects, a total of 33 POTS patients and 25 controls performed active standing tests and filled in the OHQ during their blood sample collection visit at the Clinical Research Unit at Skåne University Hospital in Malmö. Controls' active standing tests were normal. Blood samples from the remaining 15 patients with POTS were retrieved and sent to Karolinska University Hospital from local hospitals and primary care facilities why these 15 patients did not complete the OHQ and active standing tests at the time of their blood sample collection visit.

Measurement of GPCR Activity

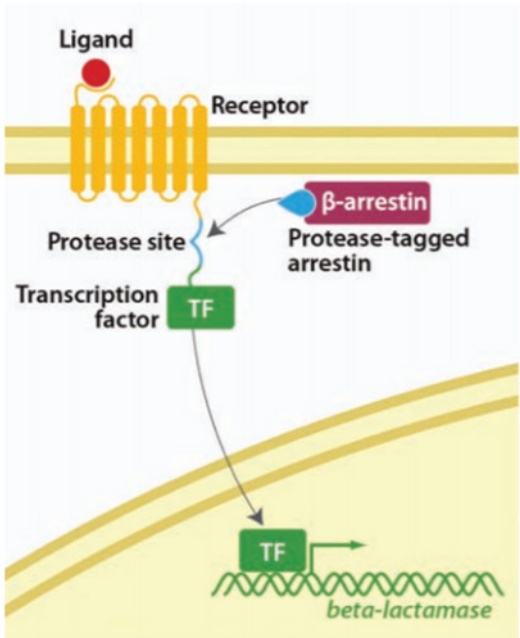


Figure 6. FRET based method for measuring G-protein coupled recerptor activity (GPCR). A protease cleavage site and a transcription factor (TF) are added to the GPCR. Beta-arrestin is in turn tagged to a protease. A conformational change in the GPCR due to ligand binding leads to the recruitment of beta-arrestin. When the beta-arrestin is recruited the protease and transcription factor come to close proximity causing the TF to be cleaved. The TF then enters the nucleus and initiates transcription of beta-lactamase which subsequently cleaves the FRET substrate, resulting in two different emission wavelengths. Reprinted from Hanson, Bonnie J et al. "A homogeneous fluorescent live-cell assay for measuring 7-transmembrane receptor activity and agonist functional selectivity through beta-arrestin recruitment." Journal of biomolecular screening vol. 14,7 (2009): 798-810. doi:10.1177/1087057109335260. Licensed under CC BY-NC-ND.

Human embryonic kidney 293 cells overexpressing specific G-protein-coupled receptors (GPCR) (adrenergic α1 receptor, adrenergic β2 receptor, cholinergic muscarinic type 2 receptor, or opioid receptor-like 1) were treated with sera from

POTS and controls. Receptor activity was analysed by a fluorescence resonance energy transfer (FRET)- method (Tango GeneBLAzer, Thermo Fisher Scientific) based on a β_2 – arrestin-linked transcription factor driving transgenic β lactamase transcription. GPCR activity was determined as the ratio between emission of cleaved and noncleaved FRET substrate. The FRET-based method has previously been described in detail (76). The selection of the ADRB2 over the ADRB1 stemmed from previous findings (24), which revealed that the combination of α1 and β2 adrenergic receptors, provides the highest discriminative efficacy regarding patients with POTS (≈94%). Please see figure 6, for a schematic overview of the Tango assay.

Statistical analysis paper 2

OHQ scores were compared between POTS patients with serum activation levels above or below the median, using independent samples t-test. Receiver operating characteristic curves (ROC) were constructed to analyze the predictive value of GPCR activity for POTS. A logistic model with all 4 GPCRs as POTS predictors was performed and a predicted value for every individual was calculated.

The activation of the GPCRs was transformed into z-scores (standard deviations from the mean), log-transformed and analyzed in relation to both the composite and individual OHQ scores using age-adjusted linear regression models. Additionally, the association between GPCR activity and OHQ scores was further examined in linear regression models including changes in HR and SBP after three minutes of active standing as additional covariates.

Paper 3

A total of 61 HF patients (NYHA class I-III) from the HARVEST study and 60 control patients from the SYSTEMA cohort (inclusion between 2014-2017) performed HUT, with simultaneous non-invasive hemodynamic monitoring (SBP and HR) and cerebral oximetry. Control patients had a normal HUT response and did not have any heart disease. Hemodynamic variables and SctO2 values were collected in supine position and after 1, 3 and 10 minutes of HUT. Delta SBP, HR and SctO2 were defined as the difference between parameters in supine position and after 10 minutes of HUT.

Statistical analysis paper 3

Group differences in continuous variables between HF patients and controls were analyzed using independent-samples t-test, whereas within-group changes during HUT were assessed using paired-samples t-test. We compared the proportion of patients with SctO2 levels below 65% (the lower normal limit for subjects with a normal HUT response) and below 60% (the threshold at which patients typically experience syncope) (65) after 10 minutes of HUT between HF patients and

controls, using Pearsons Chi2-test. Associations between HF diagnosis and SctO2 levels in supine position and after 10 min of HUT were studied using multivariable-adjusted linear regression models, including age, sex, smoking, diabetes, SBP and HR in supine position and after 10 min of HUT. Also, to study associations between SctO2 and age, sex, smoking, diabetes, SBP, and HR in HF patients and controls, univariable linear regression was performed separately in each group.

Paper 4

Paper 4 was based on the SYSTEMA cohort, including patients who had been examined between 2016-2020. Patients with VVS, OH, and those showing a normal response to the HUT (negative HUT) were selected based on clinical interpretations of their HUT responses, after excluding individuals with missing data or inconsistent cerebral oximetry signals. The study included 139 patients with spontaneous or NTG induced VVS, 121 with OH (39 with classical OH and 82 with delayed OH), and 82 patients who displayed a normal response to HUT (negative HUT) following NTG administration. Patients who exhibited abnormal responses to carotid sinus massage were excluded from the study.

VVS was defined as the reproduction of syncope associated with a pronounced pattern of hypotension, bradycardia, or asystole. OH was defined as a sustained decrease in SBP of at least 20 mmHg and/or a decrease in DBP of at least 10 mmHg, or an SBP lower than 90 mmHg (35).

Patients diagnosed with VVS, OH, and those with a negative HUT were categorized into three age groups: under 30 years, 30–60 years, and over 60 years. This grouping is based on previous findings that indicate a bimodal distribution of the first incidence of syncope, with peaks occurring in individuals under 30 and over 60 years of age (42).

Statistical analysis paper 4

Group differences in SctO2 and SBP in supine, at 3 and 10 min of HUT, 30s before syncope (i.e. at presyncopal phase) and during syncope, were studied according to age (<30, 30-60, >60 years), using one-way ANOVA after testing for the homogeneity of variance. If the homogeneity of variance had a significance level of <0.05, Welch test was performed instead. Either Tukey's multiple comparisons test or Games–Howell comparisons post-hoc test were performed to study the differences between the three age groups.

Univariable linear regression models were used to explore the relationship between SctO2 or SBP (dependent variables) and age (independent variable). Additionally, multivariable-adjusted linear regression models were constructed, with adjustments for sex and concurrent SBP or SctO2, depending on the

dependent variable selected. Specifically, when SctO2 was the dependent variable, adjustments were made for sex and concurrent SBP. Conversely, if SBP was the dependent variable, the model adjusted for concurrent SctO2.

Paper 5

A study protocol of an exercise training program for POTS was constructed. We aim to recruit a total of 200 patients diagnosed with POTS. The study will be conducted as a randomized cross-over study. Study participants will be divided into two groups (Group A and B) and assigned randomly using the Research Randomizer tool by Urbaniak, G. C., & Plous, S. (2013) available at http://www.randomizer.org/. Group A will begin the training program first, and upon completion, Group B will commence the same program (see Fig. 7 for a schematic overview of the study design). During periods when not actively involved in the training program, members of the "non-active" group will be encouraged to maintain physical activity at home, according to their own abilities ("at home training").

The study will include individuals 18 years or older diagnosed with POTS who have provided written informed consent to participate. Exclusion criteria include patients with myalgic encephalomyelitis or physical disabilities that prevent them from being able to perform the training. Recruitment started in November 2022. Patients will be consecutively asked for participation in the study until the target sample size has been reached. POTS symptoms will be evaluated using MAPS, OHQ and SF-36. Hemodynamic parameters will be evaluated by active standing tests and maximum exercise capacity evaluated by bicycle exercise test. Symptoms, hemodynamic parameters, and exercise capacity will be assessed before and after a 16-week training program.

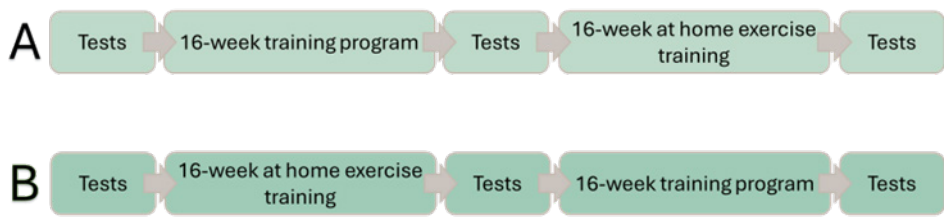


Figure 7. Schematic overview of study design. Study participants will randomly be divided into two groups (Group A and B). Group A will begin the 16-week training program first, and upon completion, Group B will commence the same. During periods when not actively involved in the training program, members of the "non-active" group will be encouraged to maintain physical activity at home, according to their own abilities ("at home exercise training"). Symptoms, hemodynamic parameters, and exercise capacity will be assessed before and after a 16-week training program.

The 16-week training program includes two weekly sessions at the clinic, each lasting up to 60 minutes. Initially, training sessions will last approximately 10 minutes, with plans to gradually increase the duration each week as tolerated by the patient. In addition to these sessions, patients are encouraged to perform specific exercises at home once per week. Depending on the severity of their POTS symptoms, training may be conducted on specialized exercise bicycles either in a supine or upright position. All exercises will be supervised by physiotherapists with special interest in POTS.

Statistical analysis paper 5

Power calculations were performed. Using an alpha risk of 0.05 and a beta risk of 0.20 with an estimated follow-up loss of 25 %, we estimated that we would need approximately 100 patients in each group to detect improved symptoms by 10 %. This calculation was based on previous results from studies on exercise training programs in POTS (27, 29, 32).

After assessing normality and homogeneity of variance of the data, either parametric or non-parametric statistics will be used for the within and between-group analysis. Within-group results will be analyzed with paired samples t-test (or Wilcoxon test for non-parametric data) whereas between-group results will be analyzed using independent samples t-test (or Mann-Whitney u-test for non-parametric data).

Summary of results

The complete results are presented in the papers at the end of this thesis. The most important findings are briefly described below.

Paper 1

Mean age was similar among POTS and control patients (29.1± 9.5 years and 29.4± 9.0, respectively). A total of 26 patients were female and eight were male in each group. During HUT, seven patients developed VVS prior to NTG administration and another eight patients developed VVS after NTG administration. Dizziness during HUT was reported by 25 POTS patients. By the study design, none of the controls experienced VVS during passive HUT. However, following NTG administration, 23 control patients experienced VVS.

In this study, we found that POTS patients had lower minimum SctO2 values, before possible reflex activation, compared to controls (65.4 ± 5.6 vs 68.2 ± 4.2, p = 0.023). Also, the difference between supine SctO2 and minimum SctO2 (delta SctO2 minimum) was greater in POTS compared with controls (5.7 ± 2.9 vs 4.3 ± 2.1, p = 0.028). We found no difference in SctO2 in supine position, and after 1, 3 and 10 minutes of HUT between POTS and controls. However, the proportion of patients with SctO2 < 65 % at 1 and 3 minutes was higher among POTS compared to controls (29.4 vs 8.8 % at 1 minute and 30.3 vs 9.1% at 3 minutes respectively). For further details, see table 1. Women had lower SctO2 compared to men during all timepoints except after 10 min of HUT in the POTS group. No sex differences in SctO2 were observed in the control group.

Table 1. Cerebral tissue oxygenation in POTS and control patients during head-up tilt test. Continuous variables are expressed as mean (standard deviation), whereas dichotomous variables are presented as percentages of total within each group. P-values are from independent samples t-test for continuous data and Pearson's χ2 test for dichotomous data. Abbreviations: POTS, postural orthostatic tachycardia syndrome; pp, percentage points; SctO2, cerebral tissue oxygen saturation. The delta value indicitates the decrease in SctO2 from supine value to lowest measured value prior to reflex activation (SctO2 minimum). Table is adapted from Kharraziha, I et al. "Monitoring of cerebral oximetry in patients with postural orthostatic tachycardia syndrome." Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology vol. 21,10 (2019): 1575-1583. doi:10.1093/europace/euz204. Licensed under CC BY-NC.

SctO2	POTS	Control patients	P-value
Supine (pp)	71.1 (4.6)	72.5 (3.4)	0.150
1 min (pp)	68.4 (5.9)	70.4 (4.2)	0.111
3 min (pp)	67.8 (6.0)a	69.7 (4.2)a	0.139
10 min (pp)	67.8 (5.5)b	69.4 (4.3)a	0.193
Minimum (pp)	65.4 (5.6)	68.2 (4.2)	0.023
Delta (pp)	5.7 (2.9)	4.3 (2.1)	0.028
<0.65 1 min (%)	29.4	8.8	0.031
<0.65 3 min (%)	30.3a	9.1a	0.030
<0.65 10 min (%)	21.2b	9.1a	0.170
<0.65 minimum (%)	35.3	23.5	0.287

a Missing values due to syncope <10 min or inadequate SctO2 signal quality = 1.
b Missing values due to syncope <10 min or inadequate SctO2 signal quality = 4.

After linear regression, we found that the decrease in SBP from supine to minimum SBP (delta SBP minimum) and increase in HR from supine to HUT value at 3 minutes (delta HR 3 minutes) was associated with a more pronounced SctO2 decrease in POTS but not controls (B=0.1170; p = 0.004 and B=0.0809; p = 0.022 respectively). However, there were no associations between SBP or HR and SctO2 in the supine position or during HUT at 1, 3, or 10 minutes or at SctO2 minimum. Figure 8 demonstrates an example of a POTS patient during HUT, showing a reciprocal change in SctO2 and HR. Finally, POTS patients who experienced syncope or dizziness during HUT did not have a significantly lower SctO2 compared to POTS patients who did not experience these symptoms.

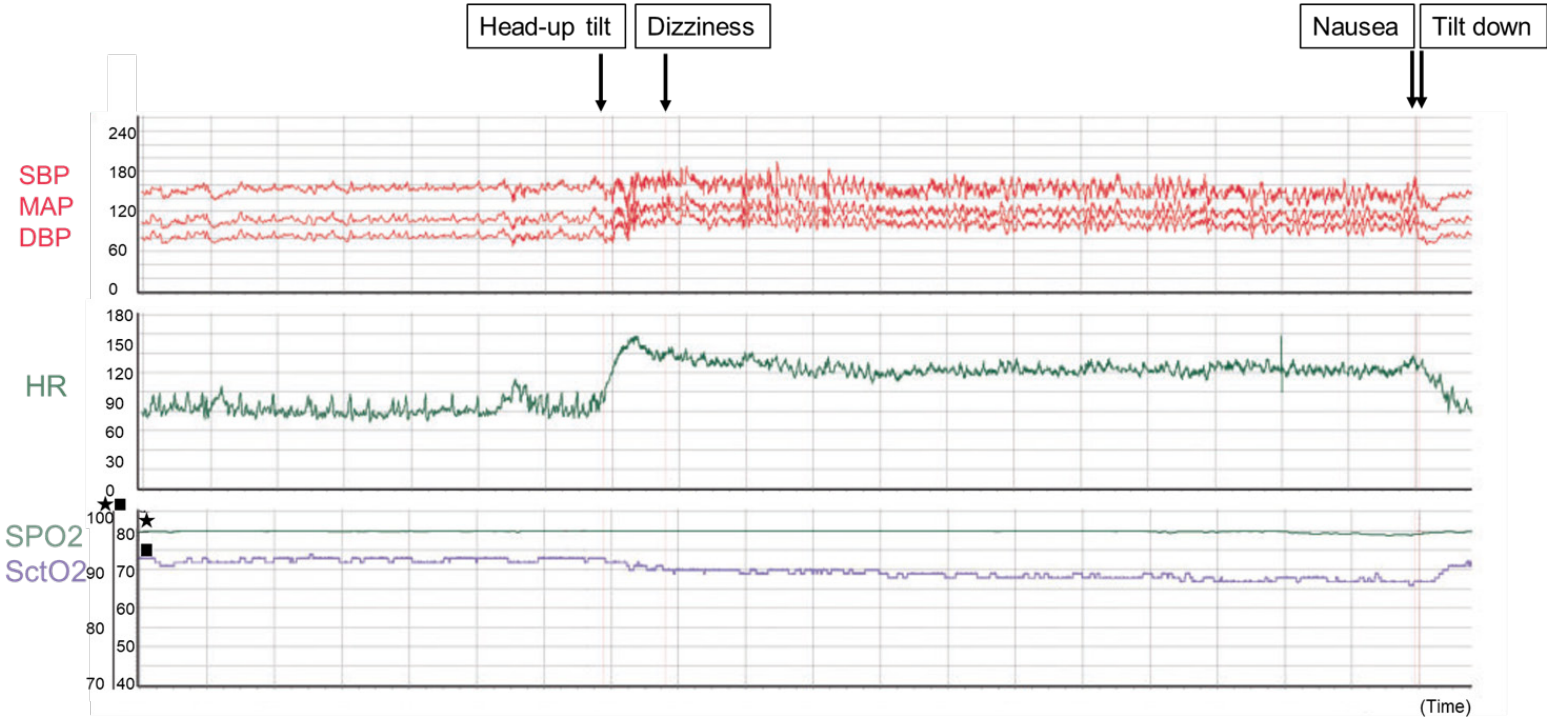


Figure 8. Example of a POTS patient during head-up tilt test. The diagram shows changes in HR, blood pressure, SPO2 and SctO2 during head-up tilt test. Note the inverse correlation between HR and SctO2 in this POTS patient. Abbreviations: HR: heart rate; POTS: postural orthostatic tachycardia syndrome; SPO2, peripheral oxygen saturation; SctO2: cerebral tissue oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. Adapted from Kharraziha, I et al. "Monitoring of cerebral oximetry in patients with postural orthostatic tachycardia syndrome." Europace: European pacing, arrhythmias, and cardiac electrophysiology journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology vol. 21,10 (2019): 1575-1583. doi:10.1093/europace/euz204. Licensed under CC BY-NC.

Paper 2

The average age was 28.6±10.5 years in the POTS group and 30.7±8.6 years in the control group. Additionally, 91.7% of the POTS patients were women, compared to 84% of the controls. Receptor activity was significantly higher in POTS patients compared to controls for all four receptors (Figure 9). The combined analysis of all four receptors yielded an area under the curve (AUC) of 0.88 (confidence interval 0.80–0.97, P<0.001), as shown in Figure 10. When analysing receptors individually, the AUCs were 0.72 (0.58–0.85, P<0.001) for ADRA1, 0.76 (0.64–0.88, P<0.001) for ADRB2, 0.73 (0.60–0.87, P<0.001) for CHRM2, and 0.75 (0.62–0.88, P<0.001) for OPRL1, as detailed in Figure 10.

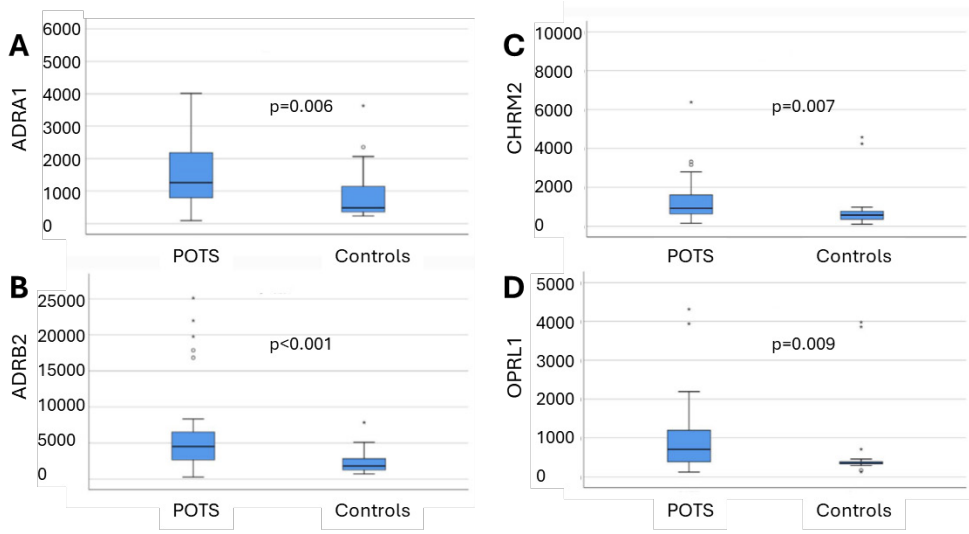


Figure 9. Receptor activation in POTS and control patients. GPCR activity is represented as the ratio between emitted light from cleaved and non-cleaved substrate, is depicted on the y-axis. A: ADRA1 activation levels. B: ADRB2 activation levels. C: CHRM2 activation levels. D: OPRL1 activation levels.

P values indicate the difference between mean values, calculated using independent samples t-test for the log-transformed receptor activity data. Please note that by design, the figures do not display an extreme outlier in the POTS group with ADRA1 activity of 14.838 and CHRM2 activity of 26.709. Abbreviations: POTS, postural orthostatic tachycardia syndrome, GPCR, G-protein coupled receptor; ADRA1, (adrenergic α1 receptor); ADRB2, adrenergic β2 receptor; CHRM2, cholinergic muscarinic 2 receptor; OPRL1, opioid-receptor-like 1.

Adapted from Kharraziha, I et al. “Serum Activity Against G Protein-Coupled Receptors and Severity of Orthostatic Symptoms in Postural Orthostatic Tachycardia Syndrome.” Journal of the American Heart Association vol. 9,15 (2020): e015989. doi:10.1161/JAHA.120.015989. Licensed under CC BY-NC-ND.

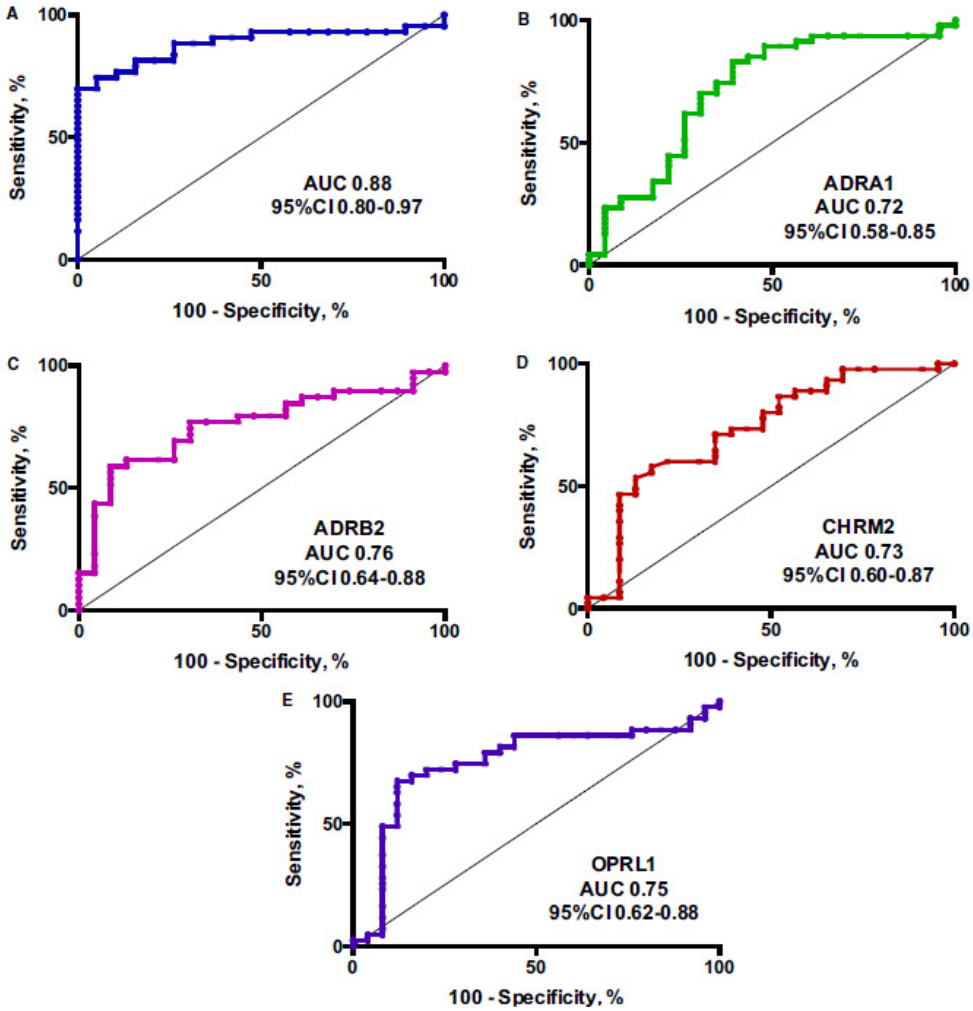


Figure 10. Receiver operating characteristic (ROC) curves of receptor activity for predicting POTS (n=73). ROC curves are shown for all four GPCRs combined (A) and individually (B through E). A: ROC curve for all four GPCRs (ADRA1, ADRB2, CHRM2 and OPRL1). B: ROC curve for ADRA1. C: ROC curve for ADRB2. D: ROC curve for CHRM2. E: ROC curve for OPRL1 in diagnosing POTS. Abbreviations: POTS, postural orthostatic tachycardia syndrome, GPCR, G-protein coupled receptor; ADRA1, (adrenergic α1 receptor); ADRB2, adrenergic β2 receptor; CHRM2, cholinergic muscarinic 2 receptor; OPRL1, opioid-receptor-like 1; AUC, area under the curve. Reprinted from Kharraziha, I et al. "Serum Activity Against G Protein-Coupled Receptors and Severity of Orthostatic Symptoms in Postural Orthostatic Tachycardia Syndrome." Journal of the American Heart Association vol. 9,15 (2020): e015989. doi:10.1161/JAHA.120.015989. Licensed under CC BY-NC-ND.

The OHQ composite score was significantly higher in POTS patients who had serum ADRA1 activation above the median (P=0.043), but not for ADRB2, CHRM2, or OPRL1.

Serum ADRA1 activation was associated with the OHQ composite score (β=0.77, OHQ points per SD of receptor activity; P=0.009), with no significant findings among controls (P=0.953). This association persisted even after adjusting for changes in HR and SBP after 3 minutes of active standing (P=0.031) (see table 2). ADRA1 activation was also related to symptoms experienced during prolonged standing (P=0.037) and during both short (P=0.042) and long (P=0.001) walking periods.

Table 2. G.protein coupled receptor activity in relation to symptom severity. Linear regression was performed to evaluate the association between symptom severity (assessed from composite OHQ score) and receptor activity, with all analyses adjusted for age. All analysis were adjusted for age.The "adjusted p-value" reflects adjustments for the increase in heart rate from baseline to three minutes, as well as the decrease in systolic blood pressure from baseline to three minutes during orthostatic tests. B-values are presented as standard deviations from the mean. Abbreviations: OHQ, orthostatic hypotension questionnaire; ADRA1, adrenergic receptor alpha 1; ADRB2, adrenergic receptor beta 2; CHRM2, cholinergic receptor muscarinic 2; OPRL1, opioid receptor-like 1). Adapted from Kharraziha, I et al. "Serum Activity Against G Protein-Coupled Receptors and Severity of Orthostatic Symptoms in Postural Orthostatic Tachycardia Syndrome." *Journal of the American Heart Association* vol. 9,15 (2020): e015989. doi:10.1161/JAHA.120.015989. Licensed under CC BY-NC-ND.

Dependant variable	Independant variable	B (per SD)	P-value	Adjusted p-value
OHQ composite	ADRA1	0.768	0.009	0.031
OHQ composite	ADRB2	0.176	0.599	0.851
OHQ composite	CHRM2	0.290	0.364	0.541
OHQ composite	OPLR1	0.472	0.118	0.188

Moreover, all four receptors were related to higher scores for vision problems (ADRA1, P<0.001; ADRB2, P=0.011; CHRM2, P=0.014; OPRL1, P=0.003). Additionally, OPRL1 activity was associated with symptoms during prolonged walking (P=0.035). None of these receptors showed a significant association with the OHQ composite score in the control group.

Paper 3

Patients with HF were generally older and more often male compared to the control patients. Additional baseline characteristics are presented in table 3.

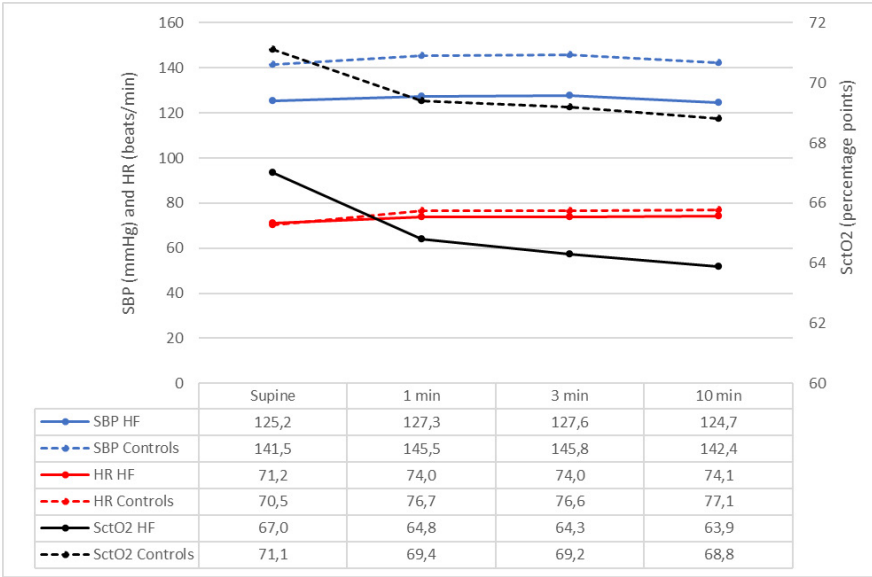
Table 3. Baseline characteristics. Abbreviations: DBP, diastolic blood pressure; EF, ejection fraction; HF, heart failure; HR, heart rate; NYHA, New York Heart Association; SBP, systolic blood pressure. Continuous variables are expressed as mean (standard deviation). Dichotomous data and NYHA classification are expressed as percentages of total within each group. Missing data: a=1 missing, b=3 missing. Adapted from Kharraziha, I et al. "Impaired cerebral oxygenation in heart failure patients at rest and during head-up tilt testing." ESC heart failure vol. 8,1 (2021): 586-594. doi:10.1002/ehf2.13128. Licensed under CC BY-NC.

Characteristic	HF (n=61)	Controls (n=60)
Age (years)	70.7 (11.0)	59.8 (11.5)
Sex (%male)	82.0	41.7
Current smoker (%)	15.0[a]	8.8[b]
Diabetes (%)	30.0[a]	15
NYHA class 1 (%)	3.3	
NYHA class 2 (%)	44.3	
NYHA class 3 (%)	52.4	
SBP (mmHg)	125.2[a] (22.9)	141.5 (19.3)
DBP (mmHg)	66.5 (12.5)	78.5 (11.6)
Heart rate (beats per minute)	71.2 (12.2)	70.5 (11.6)

Mean SctO2 was lower HF patients compared to controls, both in the supine position and after 10 minutes of HUT (Table 4 and Figure 11). During the HUT, mean SctO2 decreased significantly within both groups ($P < 0.001$). Additionally, DBP ($P = 0.038$ for HF patients; $P < 0.001$ for controls) and HR increased ($P = 0.005$ for HF patients; $P < 0.001$ for controls). The increase in HR was greater among controls compared to HF patients ($P= 0.013$) However, SBP remained unchanged during HUT in both HF patients ($P = 0.794$) and controls ($P = 0.527$).

Table 4. Cerebral tissue oxygenation in heart failure patients and controls during head-up tilt test. Continuous variables are expressed as mean (standard deviation), whereas dichotomous variables are presented as proportions in %. P-values are from independent samples t-test for continuous data and Pearson's $\chi 2$ test for dichotomous data. Delta Scto2 indicates the decrease in SctO2 from supine to 10 minutes of HUT. Abbreviations: HF, heart failure; HUT, head up tilt; SctO2, cerebral tissue oxygenation. Adapted from Kharraziha, I et al. "Impaired cerebral oxygenation in heart failure patients at rest and during head-up tilt testing." ESC heart failure vol. 8,1 (2021): 586-594. doi:10.1002/ehf2.13128. Licensed under CC BY-NC.

Parameter	HF (n=61)	Controls (n=60)	P-value
SctO2 supine	67.0 (5.0)	71.1 (3.5)	<0.001
SctO2 10 minutes HUT	63.9 (4.5)	68.8 (3.1)	<0.001
Delta SctO2 10 minutes HUT	3.1 (2.1)	2.2 (2.0)	0.026
SctO2 10 minutes HUT < 65 %	59 %	8.3 %	<0.001
SctO2 10 minutes HUT < 60 %	14.8 %	0 %	0.002



	Supine	1 min	3 min	10 min
SBP HF	125,2	127,3	127,6	124,7
SBP Controls	141,5	145,5	145,8	142,4
HR HF	71,2	74,0	74,0	74,1
HR Controls	70,5	76,7	76,6	77,1
SctO2 HF	67,0	64,8	64,3	63,9
SctO2 Controls	71,1	69,4	69,2	68,8

Figure 11. Changes in systolic blood pressure, heart rate, and cerebral tissue oxygenation during head-up tilt test in heart failure patients and controls. Mean SctO2 for HF patients is represented by solid black lines, while the controls are shown by dashed black lines. The figure shows changes in SctO2 in relation to SBP (in blue) and HR (in red) throughout the head-up tilt test. Abbreviations: SctO2, cerebral tissue oxygenation; SBP, systolic blood pressure; HR, heart rate; HF, heart failure. Reprinted from Kharraziha, I et al. "Impaired cerebral oxygenation in heart failure patients at rest and during head-up tilt testing." ESC heart failure vol. 8,1 (2021): 586-594. doi:10.1002/ehf2.13128. Licensed under CC BY-NC .

The decline in SctO2 from the supine position to 10 minutes of HUT was more pronounced in HF patients than in controls (P = 0.026) (Table 4 and Figure 11). Furthermore, a higher proportion of HF patients had SctO2 levels below 65% and 60% after 10 minutes of HUT compared to controls (Table 4).

After linear regression, we found that HF was associated with significantly lower SctO2 in the supine position (- 2.457 percentage points, P = 0.023) and after 10 minutes of HUT (- 2.597 percentage points, P = 0.007), even after adjusting for age, sex, smoking, diabetes, supine SBP, and HR. In HF patients, older age and higher supine HR were linked to lower SctO2 in the supine position. Additionally, older age, smoking, and lower SBP were factors associated with lower SctO2 after 10 minutes of HUT (Table 5). No significant associations were found between SctO2 and age, sex, smoking, diabetes, SBP, or HR in the control group.

Table 5. Association between cerebral tissue oxygenation and clinical profile in heart failure patients. Univariable linear regression among heart failure patients including SctO2 as dependent variable and age, sex, smoking, diabetes, SBP, and HR as independent variables. Abbreviations: HR, heart rate; HUT, head up tilt; SBP, systolic blood pressure; SctO2, cerebral tissue oxygen saturation. Adapted from Kharraziha, I et al. "Impaired cerebral oxygenation in heart failure patients at rest and during head-up tilt testing." ESC heart failure vol. 8,1 (2021): 586-594. doi:10.1002/ehf2.13128. Licensed under CC BY-NC.

Dependant variable	Independant variable	Beta	P-value
SctO2 supine	Age	-0.168	0.003
SctO2 supine	Sex	1.351	0.419
SctO2 supine	Current smoker	-3.170	0.079
SctO2 supine	Diabetes	-1.389	0.327
SctO2 supine	SBP supine	0.036	0.200
SctO2 supine	HR supine	-0.143	0.005
SctO2 10 min HUT	Age	-0.165	0.001
SctO2 10 min HUT	Sex	1.431	0.342
SctO2 10 min HUT	Current smoker	-3.261	0.045
SctO2 10 min HUT	Diabetes	-0.722	0.574
SctO2 10 min HUT	SBP 10 min HUT	-0.061	0.011
SctO2 10 min HUT	HR 10 min HUT	-0.085	0.083

Paper 4

The OH patients were older compared to those with VVS and patients with negative HUT. Baseline characteristics are shown in table 6. Of the VVS patients, 26 experienced spontaneous syncope, while the remaining 113 patients had NTG induced VVS.

Table 6. Baseline characteristics. Continuous variables are presented as mean (standard deviation). Dichotomous data are shown as percentages within each group. P-values indicate overall differences determined by one-way ANOVA for continuous variables and Pearson's chi-2 test for dichotomous variables. *Ischemic heart disease = previous acute myocardial infarction, unstable angina, percutaneous coronary intervention and/or coronary artery bypass graft surgery. Reprinted from Kharraziha, I et al. "The Influence of Age on Cerebral Tissue Oxygenation in Vasovagal Syncope and Orthostatic Hypotension." Journal of clinical medicine vol. 11,15 4302. 25 Jul. 2022, doi:10.3390/jcm11154302. Licenced under CC BY.

Characteristics	VVS (n=139)	OH (n=121)	Negative HUT (n=82)	P-value
Age (years)	45.1 (17.1)	61.4 (19.2)	44.5 (18.2)	<0.001
Women (%)	59.7	49.6	61.0	0.165
Hypertension (%)	20.7	36.4	12.9	<0.001
Ischemic heart disease* (%)	4.3	6.6	6.1	0.701
Stroke (%)	1.4	11.6	2.4	<0.001
Heart failure (%)	1.4[a]	4.4	6.1	0.090
Atrial fibrillation (%)	1.4	12.4	2.4	<0.001
Diabetes mellitus (%)	6.5	12.4	9.8	0.260
Current smoker (%)	10.8	14.9	27.2[a]	0.006
Resting HR (beats/min)	66.3 (11)	70.5 (11.1)	70.6 (11.5)	0.004
Resting SBP (mmHg)	131.3 (16.4)	142.0 (22.6)	134.0 (17.7)	<0.001

a= 1 missing. Abbreviations: HR, heart rate; HUT, head-up tilt test; VVS, vasovagal syncope; OH, orthostatic hypotension; SBP = systolic blood pressure;

In VVS patients, age showed an inverse association with SctO2 during the presyncopal phase (β = -0.096 per year; p = 0.001), adjusted for concurrent SBP and sex. However, the decrease in SctO2 from the supine to the presyncopal phase (delta presyncopal phase) was not age-related after adjustments for sex and SBP (β = 0.026 per year, p = 0.315). SBP was consistently associated with age in VVS patients except for SBP during the presyncopal phase.

For OH patients, older age associated with lower SctO2 in supine position and at 3 minutes, and 10 minutes of tilt, minimum SctO2 and during the presyncopal phase. Yet, the association between delta SctO2 presyncopal phase and age did not hold after adjusting for sex and concurrent SBP (β = 0.070 per year, p = 0.052). SBP in OH patients associated with age, at all timepoints, except during the presyncopal phase.

For patients with a negative HUT, age was inversely related to SctO2 in the supine position (β = -0.085 per year; p = 0.010), with adjustments for sex and SBP. No further significant associations between SctO2 and age were observed in this group during HUT. SBP levels among HUT negative patients associated with age in the supine position and after 3 and 10 minutes of HUT (p < 0.001 for all three analyses).

Differences in SctO2 according to age (<30, 30-60, >60) are presented in figure 12. The following results are from one-way ANOVA or Welch test. SctO2 during the presyncopal phase was significantly lower in older VVS patients (p = 0.009 for all age group comparisons) compared to younger VVS patients. The decrease in

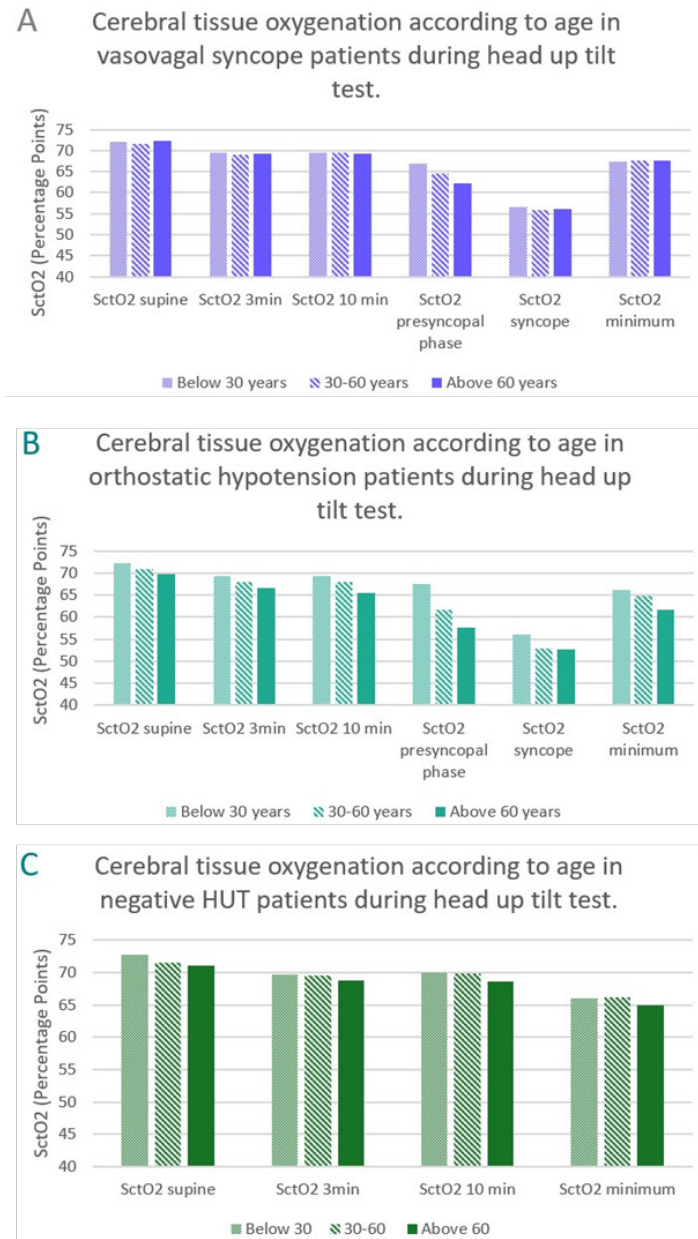


Figure 12. Cerebral tissue oxygenation according to age. SctO2 according to age groups (<30, 30-60, >60 years) in 139 VVS patients (Figure 12A), 121 OH patients (Figure 12B) and 82 negative head-up tilt patients (Figure 12C) (A). Abbreviations: HUT = head-up tilt test; OH = orthostatic hypotension; SctO2 = cerebral tissue oxygenation; VVS = vasovagal syncope. Reprinted from Kharraziha, I et al. "The Influence of Age on Cerebral Tissue Oxygenation in Vasovagal Syncope and Orthostatic Hypotension." Journal of clinical medicine vol. 11,15 4302. 25 Jul. 2022, doi:10.3390/jcm11154302. Licenced under CC BY.

SctO2 from supine to presyncopal phase was significantly more pronounced in older compared to younger VVS patients ($p = 0.003$). No other significant differences were found at other timepoints.

In OH patients, older individuals showed significantly lower SctO2 after 10 minutes of HUT, during the presyncopal phase, and at minimum SctO2 values ($p < 0.01$ for all time points). The decline in SctO2 from supine to presyncopal phase or minimum SctO2 (delta presyncopal phase or delta minimum) was greatest among older OH patients ($p=0.003$ and $p=0.018$ respectively).

No significant differences were found in mean SctO2 according to age groups among negative HUT patients, although the subgroups were relatively small.

Paper 5

Project 5 is a study protocol, why we do not have results from the exercise training program yet. The study protocol is presented in the methods section above. To date, 13 patients with POTS have been included in the program and the recruitment of additional patients is ongoing.

Discussion

In this PhD project, we assessed novel diagnostic and therapeutic methods for patients diagnosed with cardiovascular autonomic dysfunction, focusing on syncope and common syndromes of orthostatic intolerance (POTS, OH) and HF. We found that POTS patients have lower SctO2 during upright tilt, compared to patients with normal tilt test. However, the decrease only weakly correlated with heart rate increase and was not related to orthostatic dizziness and reflex syncope susceptibility. We also found that GPCR activity has a high predictive value for POTS and that receptor activity is related to POTS symptoms. Furthermore, cerebral tissue oxygenation is lower among heart failure patients at rest and during orthostatic provocation, compared to patients without heart disease and normal response to head-up tilt. We also found that older patients with VVS and OH have a lower cerebral tissue oxygenation compared to younger patients 30 seconds prior to syncope, while no age-related differences were found among control patients with normal head-up tilt during orthostatic stress. Lastly, we have constructed a study protocol for a cross-over study of a 16-week exercise program for POTS patients with the aim to explore its effect on POTS symptoms (primary outcome), hemodynamics and maximal workload (secondary outcomes).

Cerebral tissue oxygenation and POTS

Previous studies examining CBF velocity during orthostasis in POTS patients have yielded inconsistent results, with some showing higher and others lower CBF velocity compared to controls (16-19, 77). POTS heterogeneity, study size, and differences in HUT protocols may potentially explain these discrepancies.

SctO2 is determined by the oxygen content of blood, tissue diffusivity of oxygen and cerebral metabolic rate of oxygen (58). Since these factors are relatively stable over short periods of time, SctO2 might serve as useful compliment for CBF assessment. Figure 13, reprinted from Robba et al, shows the correlation between SctO2 (NIRS) and CBF velocity (transcranial doppler) in covid-19 patients admitted to the intensive care unit (63). However, a previous study (19) found that POTS patients had a significant decrease in cerebral oxygenated haemoglobin during HUT but no change in CBF velocity, which may suggest that another yet

unexplained contributing factor reducing cerebral oxygen saturation may be present in POTS.

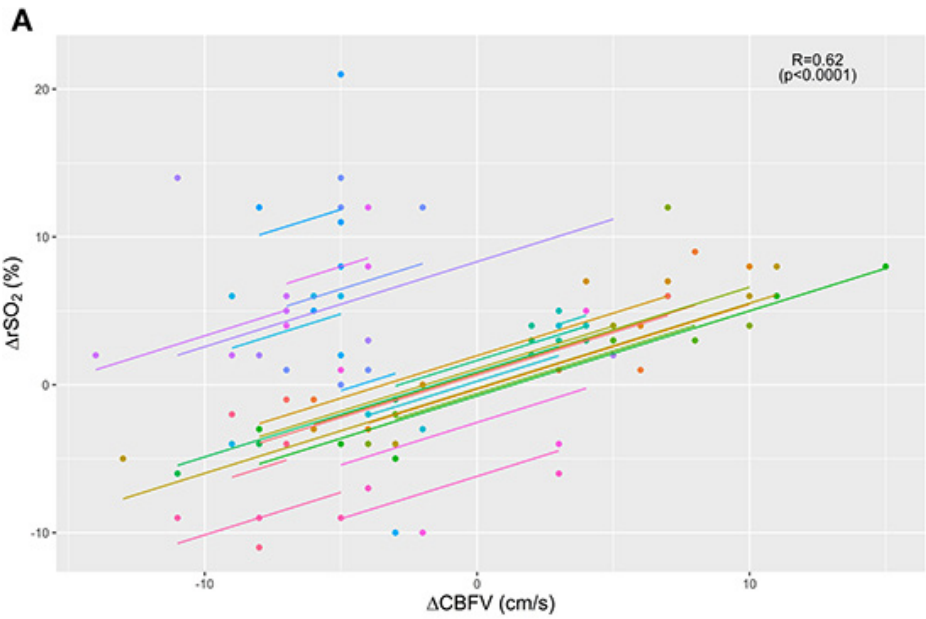


Figure 13. Scatter plot displaying the linear relationship and correlation between cerebral blood flow velocity (transcranial doppler) and total cerebral oxygenation (NIRS). Repeated measurements for each patient are represented using the same color pattern. The linear regression lines correspond to repeated measurements within individual patients. rSO2= total regional cerebral oxygenation; CBFV=cerebral blood flow velocity. Reprinted from Robba, Chiara et al. "The Use of Different Components of Brain Oxygenation for the Assessment of Cerebral Haemodynamics: A Prospective Observational Study on COVID-19 Patients." Frontiers in neurology vol. 12 735469. 20 Dec. 2021, doi:10.3389/fneur.2021.735469. Licensed under CC-BY.

Regarding cerebral autoregulation, it is possible that hemodynamic factors might influence SctO2, especially in pathological conditions where cerebral autoregulatory mechanisms are impaired. However, the increase in HR from supine to 3 minutes during upright tilt showed only a weak correlation with a more pronounced decrease in SctO2 in POTS patients. Additionally, there were no associations between HR and SctO2 at other time points. Also, the decrease in SctO2 from supine to minimum value was associated with a decrease in SBP from supine to minimum, while no associations were found between SctO2 and SBP at other timepoints during HUT in the POTS group. This lack of a consistent relationship between SctO2 and hemodynamic parameters suggests that SctO2 may be influenced by factors not directly related to hemodynamics. Thus, observations of lower SctO2 during HUT in POTS patients compared with

controls, underscore the hypothesis that the reduced SctO2 in POTS during HUT could be attributed to one or more yet unidentified factors.

Light-headedness and neurocognitive deficits (often referred to as "brain fog") are common symptoms reported by patients with POTS (12), and these symptoms do not necessarily align with hemodynamic factors. While dizziness and syncope may be symptoms of cerebral hypoperfusion, it might be assumed that SctO2 plays a role. In this study, we did not observe a significant association between reported dizziness during HUT and SctO2 levels. Additionally, there were no notable differences in SctO2 levels between POTS patients who experienced syncope during HUT and those who did not. However, it is important to interpret these results cautiously due to the small sample sizes involved.

In contrast to our findings, a previous study from the same SYSTEMA cohort, by Bachus et al (65), including patients with syncope or orthostatic intolerance, but not POTS, reported a more pronounced decline in SctO2 in those who fainted during HUT compared to those who did not. Typically, the normal range for SctO2 is reported as 60–80% (59). However, data from the earlier study (65) indicated that subjects with a normal HUT response maintained SctO2 levels above 65%. Therefore, we adopted a more stringent threshold, considering SctO2 levels below 65% as abnormal for this study. The specific SctO2 threshold at which dizziness and other symptoms might occur likely varies depending on their underlying causes. This variability underscores the need for further research to better understand the relationship between SctO2 levels and symptom manifestation during orthostatic challenges.

Interestingly, SctO2 levels tended to be lower in women within the POTS group, whereas no gender differences in SctO2 were observed in the control group. This contrasts with previous findings from the study by Bachus et al, where SctO2 levels were generally slightly higher in women at baseline (65). However, our results on gender differences should be interpreted with caution since the study only included 8 male patients. The potential relationship between gender differences in SctO2 levels and the pathophysiology and female predominance in POTS warrants further investigation.

The observation of lower SctO2 during HUT in POTS patients in this study raises questions about whether this is a consequence or partial cause of POTS symptoms. Although the differences in SctO2 observed are small and their clinical significance remains unclear, the potential benefits of increasing SctO2 should not be dismissed. Given that POTS is likely a heterogeneous condition with several overlapping subtypes, such as hyperadrenergic and hypovolemic types, future studies should explore these potential phenotypes in relation to SctO2 during tilt. Such research could provide valuable insights into the underlying pathophysiology of POTS.

Cerebral oximetry and age-related differences among VVS, OH and negative HUT patients

In addition to POTS, cerebral oximetry was also applied in VVS and OH patients to investigate whether age was associated with different SctO2 levels during HUT. In our study, we observed that older patients with VVS and OH exhibited lower SctO2 prior to HUT induced syncope, compared with younger patients, regardless of their concurrent SBP levels. Furthermore, advanced age was linked to lower minimum SctO2 among OH patients, even though not all OH patients experienced syncope during HUT. Interestingly, no relationship was found between age and SctO2 during orthostatic provocation among patients who had a normal HUT result.

While the direct clinical implications of these findings remain uncertain, the data provide valuable insights into age-related differences in SctO2 among patients with VVS and OH. These observations could serve as a basis for future research, particularly in exploring the potential link between lower SctO2 and age-associated cognitive impairments, including amnesia during syncope. Such studies might help in developing more targeted management strategies for older patients susceptible to these conditions.

This study represents the most extensive examination to date of age-related differences in SctO2 among patients with VVS and OH. While previous research has focused primarily on cerebral circulation changes during orthostatic provocation in mostly healthy subjects, findings have been inconsistent (44-47). Our study found no link between aging and SctO2 during orthostasis in patients with negative HUT, aligning with earlier findings suggesting similar cerebral autoregulatory responses to orthostasis among older and younger healthy individuals (45, 78). Furthermore, a previous study by Sorond et al., found a similar decline in CBF velocity across younger and older individuals during orthostatic provocation (46).

In contrast, previous research has noted decreased frontal cortical oxygenation in healthy elderly compared to younger subjects (47). Variations in group heterogeneity and differences in HUT or active standing test protocols might explain the inconsistency in previous results. Our findings suggest that while aging is associated with lower SctO2 at rest, SctO2 during orthostatic provocation remains intact, potentially indicating more preserved cerebral autoregulation due to better adaptation to blood pressure fluctuations often observed in these subjects (79). It seems that cerebral autoregulation remains relatively intact in older adults (80), even though blood pressure adaptation to postural changes is often impaired with age (79).

Previous studies on age in relation to SctO2, syncope and orthostatic intolerance are sparse. In an exploratory study of the utility of cerebral oximetry in

SYSTEMA, age was not associated with SctO2 (65), however this study was small (n=54) and did not aim to assess the association between age and SctO2 in different diagnosis groups. In our research, older VVS and OH patients exhibited lower SctO2 during the presyncopal phase compared to younger patients. Lower minimum SctO2 values were also linked with increasing age among OH patients, even though not all OH patients experienced syncope. This suggests that cerebral autoregulatory mechanisms may be compromised in older VVS and OH patients, leading to lower SctO2 during standing.

Interestingly, among OH patients, the association between SctO2 and age was observed not only during the presyncopal phase but also when supine and after 3 and 10 minutes of HUT, despite higher SBP at these times. This may suggest a greater degree of cerebral autoregulation impairment in older compared to younger OH patients. It has been suggested that cerebral hypoperfusion associated with OH may contribute to the development of cognitive impairment (81). Previous research indicates that OH is commonly associated with dementia or mild cognitive impairment (82, 83). Additionally, a previous study concluded that OH in older adults is likely a risk factor for cerebral hypoxic damage and the onset of mild cognitive impairment, likely due to impaired cerebral autoregulation (82). Our findings of lower SctO2 among older OH patients might therefore underscore the need for cautious blood pressure management to avoid "overtreating" BP among these vulnerable patients.

The postural decreases in SctO2 observed suggest that older patients may have altered regulation of SctO2, potentially predisposing them to ischemic cerebral symptoms during upright postures. However, prodromal symptoms are often less evident in older individuals (42, 84), and older VVS patients are less likely to report complete or near loss of consciousness and more likely to present with unexplained falls (42). Thus, although older patients with syncope and orthostatic intolerance are more prone to cerebral ischemia when upright, their clinical presentations appear inconsistent. This inconsistency might be due to better tolerance to hypoperfusion or impaired cognition, although this remains speculative for now. Consequently, clinicians should remain vigilant for syncope as a cause when older patients present with collapses or unexplained falls, especially if the clinical history is unclear or inconsistent.

As for physiological differences, blood pressure tends to decrease more slowly, and bradycardia is typically less pronounced in older VVS patients (85). One possible explanation for why older patients in our study appear to tolerate deoxygenation during orthostatic provocation better than younger patients might be that young adults experience a loss of cerebral function after about 7 seconds of abrupt cessation in cerebral circulation—a period known as cerebral anoxia reserve time, which is notably shorter in younger individuals and longer in the elderly (84). Even though older patients seem to tolerate deoxygenation better with

respect to manifest syncope, exploring the relationship between cognitive function, amnesia of syncope, and deoxygenation during the presyncopal phase could provide valuable insights for future research.

Cerebral oximetry in heart failure patients

In addition to syncope and orthostatic intolerance, cerebral oximetry was applied in HF patients during HUT. Our study demonstrated that patients with compensated HF have lower SctO2 both in the supine position and after 10 minutes of HUT compared to hemodynamically normal controls without HF. Among HF patients, older age and higher HR were associated with lower SctO2 in supine position. Additionally, older age, smoking, and lower SBP were associated with lower SctO2 after 10 minutes of HUT in the HF group. Notably, a higher proportion of HF patients had SctO2 levels below 65% and 60%, with 15% showing values below 60% after orthostatic provocation.

In contrast to our current findings, a previous study on elderly patients with diastolic dysfunction showed a smaller decrease in SctO2 during standing compared with healthy elderly subjects (86). The conflicting results may potentially be due to the pre-test withdrawal of medications like furosemide and captopril, which might have enhanced hemodynamic responses to orthostatic challenges (87). Also, the previous study (86) included patients with predominantly diastolic dysfunction as opposed to our study which included patients with both reduced, mid-range and preserved ejection fraction. This highlights possible conflicts in results due to different study conditions and patient preparations.

Research has previously indicated that HF patients experience lower CBF during upright posture compared to controls (55), aligning with findings from our study that suggest cerebral hypoperfusion during orthostatic stress. The observation that CBF and SctO2 are reduced in HF patients compared to controls, even after adjusting for hemodynamic measurements, warrants further consideration. While the exact mechanisms causing the reduction in CBF in HF are not fully understood, they may involve low CO (55, 88) or possible vasoconstriction of the cerebral vasculature, triggered by increased sympathetic nervous system activity and the renin-angiotensin-aldosterone system (89, 90).

Additionally, common HF comorbidities like atrial fibrillation, obesity, diabetes, and sleep apnea have also been linked to reduced cerebral perfusion (91-94), potentially exacerbating the challenges in CBF regulation in HF patients.

Cerebral autoregulation and vasomotor reactivity are important for maintaining CBF (80). Vasomotor reactivity, which is the capacity of cerebral vessels to appropriately dilate or constrict in response to changes in CO2 levels in the blood

and surrounding tissues (95), plays an important role in this process. Research has shown that patients with HF often exhibit diminished vasomotor reactivity (96), which may suggest impaired cerebral autoregulation and consequently affect the maintenance of CBF and SctO2.

As previously noted, reduced CO in HF seems to play a role in the development of cerebral hypoperfusion. Since CO is determined by HR and stroke volume (97), cerebral hypoperfusion can be affected by an impaired increase in HR during orthostatic provocation. Despite a greater HR increase in controls compared to HF patients in our study, SctO2 declined in HF patients regardless of the HR response to orthostatic stress. Medications commonly used in HF, such as beta-blockers, angiotensin-converting enzyme inhibitors, diuretics, and aldosterone antagonists, could influence our findings, although differences in hemodynamic responses to orthostasis have previously been attributed more to cardiac dysfunction than to medication effects (56).

Furthermore, autonomic dysfunction could explain the abnormal cerebral oximetry responses observed in HF patients during HUT. During orthostatic stress, patients with HF have shown impaired variability in HR and blood pressure compared to those with hypertension and healthy controls (56). Additionally, studies on heart transplant recipients (98) have indicated that cerebral oxygenation-perfusion is reduced during exercise and recovery, suggesting that factors other than CO, such as autonomic dysfunction, play significant roles.

Previous research also shows that HF is associated with neural injury and loss of tissue in brain areas critical for autonomic regulation (52, 99), which might impact physiological adaptations to upright posture and contribute to the observed differences in cerebral perfusion and oxygenation between HF and controls.

Given the implications of chronically reduced SctO2 and impaired cerebral autoregulation as potential links in the pathophysiology of cognitive impairment—an independent risk factor for mortality in HF (100)—the consequences of lower SctO2 in HF patients, both short-term and long-term, warrant further exploration. This could provide deeper insights into the management and treatment strategies for HF, focusing on improving cerebral perfusion and overall patient outcomes. Previous findings from the HARVEST study showed that 29% of HF patients had signs of cognitive impairment (101), highlighting the importance of monitoring cerebral function in these patients. NIRS may potentially serve as a valuable complement in assessing cerebral function, but further research is necessary to validate its accuracy and clinical usefulness.

Assessing autoimmune activity in POTS

In paper 2, we investigated the role of a possible autoimmune involvement in POTS, by studying specific GPCRs. We found that sera from patients with POTS significantly activated four GPCRs—ADRA1, ADRB2, CHRM2, and OPRL1—more than sera from control subjects. This GPCR activity was highly predictive of POTS, as demonstrated by ROC analyses. Additionally, activity of the ADRA1 receptor is linked to the severity of orthostatic symptoms, as quantified by the OHQ scores, in patients with POTS. This association remains independent of the hemodynamic changes during active standing. This suggests that GPCR activation may play a crucial role in the symptomatology of POTS, beyond just the cardiovascular responses typically monitored.

The study supports prior research suggesting autoimmune involvement in POTS, which identified various autoantibodies (23-25, 102, 103). In the current study, instead of direct autoantibody detection, we measured the activity towards specific GPCRs using a FRET-based method.

Upon binding to GPCRs, autoantibodies can have stimulatory or inhibitory effects (6). For instance, previous studies (24, 25) found increased levels of autoantibodies against ADRA1 and ADRA1B1/2 in POTS patients compared with healthy controls. IgG from POTS patients shifted the ADRA1 dose-response curve to the right after phenylephrine administration, indicating a partial antagonistic effect on ADRA1, which could interfere with the effects of endogenous norepinephrine leading to impaired vasoconstriction and enhanced baroreceptor activation, subsequently increasing sympathetic activity. Conversely, IgG had a stimulatory effect on ADRB1/2, enhancing the response to circulating catecholamines with reflex tachycardia (24, 25). These inhibitory and stimulatory effects offer an interesting pathophysiological explanation for the cardiovascular responses observed in patients with POTS when standing.

The predictive value of GPCR activity for diagnosing POTS was assessed using the receiver operating characteristic, yielding an excellent prediction accuracy (AUC, 0.88) when all four receptors were combined. The AUC for individual receptors ranged from 0.72 to 0.76. These results suggest that GPCR activity measurement could be integrated as a diagnostic tool for POTS, although the ideal panel of receptors and precise cutoff values still need to be established. Given that POTS is likely a heterogeneous disease, further research is necessary to determine whether detecting autoimmune GPCR activity could help identify different POTS subtypes that may respond to different treatments.

In contrast to our findings of increased GPCR activity in POTS, another study from our research team in collaboration with researchers from Calgary (104), found that patients with POTS and healthy controls did not differ in their ELISA–derived autoantibody concentrations to cardiovascular GPCRs (see figure 14,

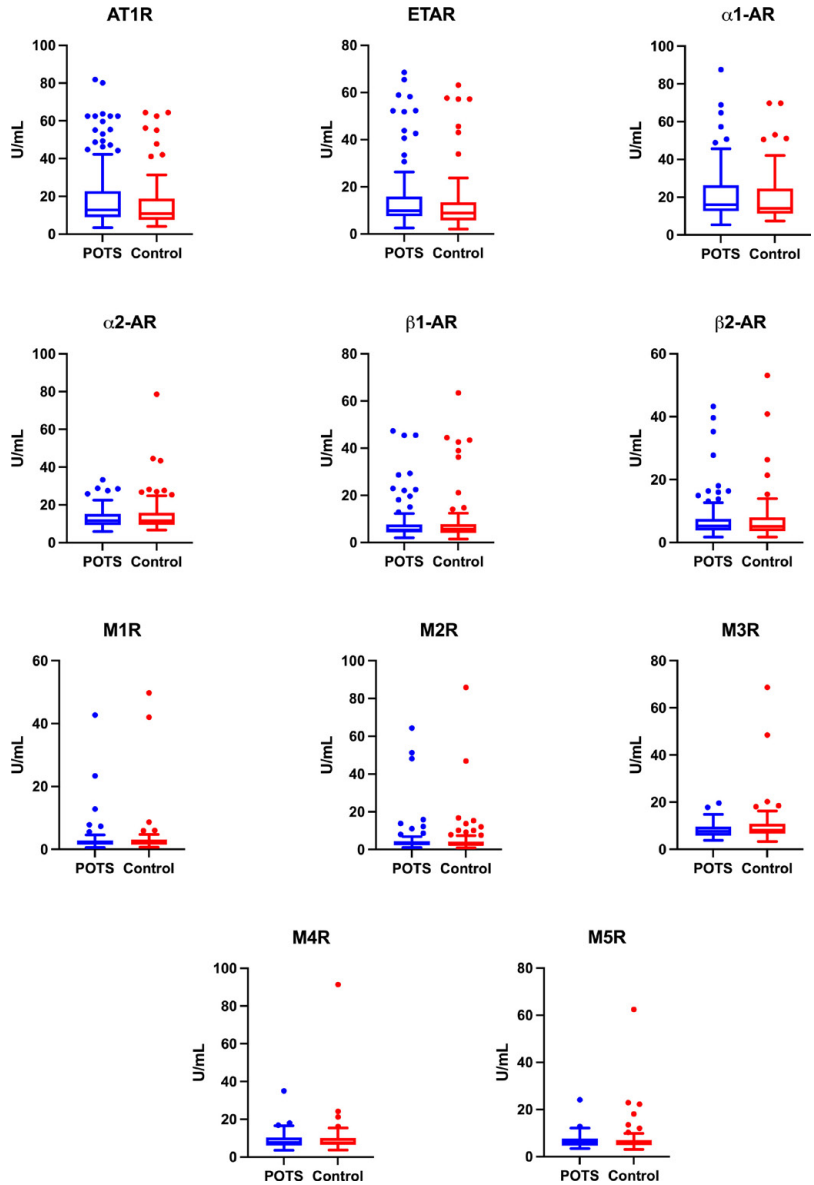


Figure 14. G-protein coupled receptor autoantibody concentrations in POTS patients and healthy controls. Autoantibody concentrations (units/mL) to 11 cardiovascular GPCRs. Data are presented as box-and-whisker plots. The box indicates the interquartile range, with the line representing the median. The whiskers extend to 1.5 times the interquartile range. Individual points beyond the whiskers represent outliers. Abbreviations: AT1R, angiotensin II receptor type 1; ETAR, endothelin receptor A; α1-AR, α1 adrenergic receptors; α2-AR, α2 adrenergic receptors; β1-AR, β1 adrenergic receptors; β2-AR, β2 adrenergic receptors; M1R, M2R, M3R, M4R, M5R, muscarinic receptors 1- 5; POTS, postural orthostatic tachycardia syndrome. Reprinted from Hall, Juliette et al. "Detection of G Protein-Coupled Receptor Autoantibodies in Postural Orthostatic Tachycardia Syndrome Using Standard Methodology." Circulation vol. 146,8 (2022): 613-622. doi:10.1161/CIRCULATIONAHA.122.059971. Licensed under CC BY-NC-ND.

reprinted from Hall et al). However, we proposed that these results did not rule out the role of autoantibodies in the pathophysiology of POTS. Of note, the study by Hall et al measured antibody concentrations in contrast to the study in the current thesis, which measured the induced activity in cells. Several previous studies have indicated that serum- or immunoglobulin G–dependent GPCR activity may be altered in the POTS population (24, 105, 106) It is crucial to investigate the mechanisms behind altered autoantibody activity, the downstream effects of this activity, and how it contributes to the pathogenesis of POTS.

A previous study by Gunning et al (23). observed a correlation between symptom severity and the presence of all 9 autoantibodies in POTS, revealing a weak correlation between autoantibody concentrations (ADRA1/2, ADRB1/2, and CHRM1–5) and severity of orthostatic symptoms. Notably, 89% of POTS patients had autoantibodies against ADRA1, while other adrenergic and muscarinic antibodies were less prevalent. Interestingly, adrenergic (α2, β1, and β2) and muscarinic receptor antibodies were often not detected unless α1 adrenergic receptor autoantibodies were present. In our study, ADRA1 activity showed a stronger association with orthostatic symptoms than ADRB2, CHRM2, and OPRL1, emphasizing ADRA1's particularly important role in POTS. Of note, Gunning et al. used ELISA for antibody detection, which, as mentioned above, only measures the level of antibodies, not the induced activity in cells.

In addition to adrenergic receptors, we included CHRM2 and OPRL1. CHRM2 autoantibodies were first identified in Chagas disease and later in dilated cardiomyopathy (6). CHRM2 was reported to have a negative chronotropic effect in cultured cardiomyocytes (107). Although CHRM2 activity was higher in POTS patients compared to controls in our study, it did not associate with symptom severity, contrasting with previous findings where symptoms correlated with all five muscarinic receptors, particularly CHRM4 (23). OPRL1, involved in pain perception (108), has not been studied previously in POTS and other orthostatic intolerance syndromes. In our study we found higher OPRL1 activity in POTS patients, and that the activity was associated with vision problems and difficulty walking for long distances, though these associations were weak. The specific roles and effects of CHRM2 and OPRL1 in POTS remain unclear.

Our study demonstrated a strong association between vision disturbances and all four GPCRs in POTS patients. Vision disturbances, a common symptom of orthostatic intolerance, may be due to the retina's susceptibility to hypoperfusion (84). However, in patients with POTS, the decrease in blood pressure when standing is generally modest or absent unless the patient experiences presyncope or syncope triggered by vasovagal reflex activation (13). The autonomic nervous system affects various ocular functions (109), which might explain why POTS patients experience disturbed vision. Also, opioid receptors are involved in regulating iris function (110) and intraocular pressure (111), which could possibly

explain the association between the OPRL1 and symptoms of disturbed vision in our study.

The association between symptoms and GPCR activity supports the hypothesis that POTS may be an autoimmune disease. However, due to the significant and potentially life-threatening complications of immunological therapies (112-114), immunotherapy should not be the primary treatment approach for POTS. The presence of GPCR autoantibodies alone is not sufficient evidence of an autoimmune cause for POTS based on existing research. However, current controlled treatment trials are exploring this possibility, with a few case reports indicating that some POTS patients respond favourably to treatments like IVIG, rituximab, and plasmapheresis (114) in highly selected patients.

Our findings pave the way for a deeper understanding of POTS as a potential autoimmune disorder and highlight the need for targeted therapeutic approaches based on immunological profiles. However, more systematic research is necessary, including randomized placebo-controlled trials, to solidify the role of immunomodulatory therapy in treating POTS. Hitherto, small randomized controlled clinical trials were not able to demonstrate a clear benefit of such therapy (115).

Exercise training as complementary treatment in POTS

Many studies have demonstrated that exercise training, as a non-pharmacologic treatment, improves the balance between the sympathetic and parasympathetic nervous systems, in patients with cardiovascular disease (116, 117). Research suggests that exercise training may normalize markers of sympathetic activity, such as those measured by microneurography, heart rate variability, or plasma catecholamine levels (117). Despite the limited effective treatment options available for POTS patients (13), exercise training has emerged as a promising approach to manage and alleviate symptoms (29, 32, 118). Implementing exercise training in clinical practice for POTS patients can offer numerous benefits but also presents specific challenges.

As previously mentioned, exercise training in POTS may improve cardiovascular fitness and enhance cardiovascular conditioning, with increased blood volume, increased heart size and improved heart rate control, as well as improvements in POTS symptoms and overall quality of life (28, 29, 119). Challenges with exercise training in POTS may be POTS heterogeneity and differences in symptom severity (requiring customized training), patient motivation and adherence to training and in some cases symptom exacerbation (post exertional malaise) (12, 29, 120, 121) .

While exercise training holds considerable therapeutic potential for improving the quality of life and symptom management in POTS patients, it requires tailored,

patient-specific approaches and significant support mechanisms to overcome these challenges. A strength with our exercise training program is that the trainings will be individualized, for each POTS patient, and training programs will be supervised by physiotherapists with special interest in POTS. Also, POTS patients will have the opportunity to perform the training in supine position if needed. The individualised and gradual training could potentially diminish the risk of symptom exacerbation. Furthermore, the training sessions will be held in groups, which could make it more cost-effective.

Another strength of this study protocol is the randomized cross-over study design, including a relatively large group of POTS patients. This randomized crossover study design will enable us to observe changes over time and facilitate comparisons both between and within study groups (122). Cross-over studies, where participants receive both the treatment and control conditions at different times (123), come with some important advantages. First, each participant serves as their own control, which may reduce bias (122). Second, cross-over trials generally allow for a fewer number of participants as each participant receives both the intervention and act as control (124). Third, cross-over studies may be considered ethical because all participants receive the intervention at some point, rather than only a placebo or less effective treatment.

An important consideration of cross-over design is the potential for carry-over effects, which are residual influences of the first treatment on outcomes measured after the second treatment (123). Our study design does not include a washout period, as our hypothesis is that the effects of exercise will persist even after the training program has ended.

Another issue that must be considered in our study is participant dropout and loss to follow-up, which are potential risks that could compromise the validity of the results and affect statistical power. To mitigate loss to follow-up, we plan to respect participants' time commitments and offer flexible testing hours. In prior studies on exercise training programs for POTS patients, dropout rates ranged from 10%, 24%, to 59% (27, 29, 32). The latter study (32), with the highest dropout rate, the training program was handed off to primary physicians without involvement from the research team. The authors of that study speculated that the high dropout rate might have been mitigated if healthcare systems had been developed where physicians and healthcare providers took greater responsibility for regularly monitoring patients, potentially leading to better adherence.

In summary, this cross-over study of an exercise training program, with the goal to include a relatively large group of POTS patients, may provide valuable information on potential benefits and how it can be implemented in clinical practice for POTS patients.

Limitations

Cerebral oximetry

Cerebral oximetry was measured with NIRS in paper 1, 3 and 4, and there are some technical limitations to discuss. First, the SctO2 levels were measured, with a penetration depth of 2.5 cm limiting information on deeper brain regions. Additionally, NIRS carries the risk of measuring saturation in overlying tissues rather than the intended cerebral regions (58). Although changes in SctO2 have been associated with circulatory responses caused by orthostatic blood pressure changes (65, 125), the shift in skin blood flow from the head to the lower body during orthostasis could also impact NIRS measurements. To mitigate the risk of measuring superficial tissue, detectors at various distances from the light source can be used (58). Factors such as motion artifacts, melanin in hair, and bilirubin in jaundice patients may also influence signals (58, 60). However, melanin content in skin does not appear to affect NIRS measurement as it is limited to the superficial part of the skin (58). Moreover, we did not analyse end-tidal carbon dioxide (CO2), which is known to affect cerebral circulation (9).

NIRS shows significant variability in individual anatomy, where baseline values vary by ~10% between individuals, making it better for intra-individual changes (58). Also, there is an issue of reproducibility in cerebral oximetry. However, according to a small study (126), NIRS measured deoxygenated Hb appears to be reproducible and may therefore be used in follow-up studies. Additionally, NIRS measures SctO2 where the probes are located and does not account for remote cerebral regions. During HUT, significant CBF redistribution was previously found in OH patients, with a reduction in frontal and an increase in postcentral areas (127). However, a previous study found that the reduced perfusion is global during orthostatic stress, however, being most pronounced in the frontal lobe (128). NIRS has also been found comparable with functional MRI and PET, which measure changes in CBF globally (129, 130)

Controls

Although our control group in paper 1, 3, and 4, consisted of patients who exhibited a normal response to orthostatic provocation, all had a history of syncope and/or orthostatic intolerance, which could influence the generalizability of the findings. It is noteworthy that even among individuals with no history of these conditions, some might still experience syncope during testing. Previous studies suggest that up to 13% of otherwise healthy individuals experience syncope during passive head-up tilt testing (131), and this percentage could be higher following NTG provocation. On the other hand, the absence of syncope during the index tilt suggests that these 'normal' individuals had normal hemodynamics and no syncope on that particular day, with none showing signs of POTS or heart disease.

Sample size

A limitation with papers 1 and 2 is the small sample size, which necessitates validation of our results in larger cohorts. Also, in paper 1, the subgroup analysis for comparing SctO2 according to sex as well as comparisons between symptoms (dizziness and syncope) during the HUT in relation to SctO2 levels were even smaller, and these results must be interpreted with caution.

OHQ,

The OHQ that was used in paper 2 and 5, has been validated for OH but not specifically for POTS (71). Patients with POTS often experience a broader range of symptoms, such as cognitive impairments, gastrointestinal issues, and unexplained pain, which the OHQ does not specifically address (74). In paper 5, MAPS will be used in addition to the OHQ to be able to detect more POTS specific symptoms.

Paper specific limitations

Paper 1: As noted earlier we did not analyse end-tidal CO2. A previous study found that a subset of POTS patients exhibited postural hyperventilation and hypocapnia during HUT, which caused reduced CBF velocity and consequent light-headedness (132). However, another previous study found that normocapnic POTS patients had lower SctO2 levels compared to controls (18), indicating that the cerebral circulation may be impaired even with normal CO2 levels. Another

limitation was that we could not determine whether the lower SctO2 observed during HUT was a cause or a consequence of POTS.

Paper 2: We did not measure ADRB1 activity as outlined in the methods section. Moreover, many POTS patients were on heart rate-regulating or vasoactive medications during both the symptom questionnaire assessment and blood sample collection. Despite this, these patients still reported significant symptoms as reflected in the OHQ scores. Also, as mentioned earlier, our study measured receptor activity instead of antibodies. The conformational changes observed in the GPCRs could potentially be caused by factors other than autoantibodies. Nonetheless, given that several previous studies have identified antibodies against these GPCRs (23-25), it might be reasonable to speculate that the heightened activity in these receptors is a result of autoimmune responses.

Paper 3: There is potential for selection bias in paper 3, as patients from the HARVEST-Malmö study were invited to perform HUT. It is possible that healthier HF patients might be more likely to participate in such a study. However, including potentially healthier patients might provide insights that are more relevant from a preventive standpoint than if only severely affected patients were included.

There are some issues with age differences among study groups that need to be discussed. Although efforts were made to match the ages of participants, the HF patients were generally older and more often male compared to the control subjects. Previous research has shown that brain tissue oxygen partial pressure in the barrel cortex of healthy mice decreases with age (133), and age-related arterial stiffness has been observed to reduce CBF (134, 135), both of which could impact our findings. Furthermore, previous research suggests that age-related changes in cerebral cortex myelination result in greater dispersion of infrared light passing through the brain, which may account for the influence of age on baseline SctO2 levels (136). Despite these issues, the differences in SctO2 between the groups remained statistically significant even after adjusting for age in the linear regression models, suggesting that HF patients exhibit lower cerebral saturation independent of age.

Moreover, the use of medications that affect the ANS and hemodynamic response was common among the HF group but not in the controls, which could have influenced the results.

Paper 4: Similar to paper 3, there were age differences among study groups in paper 4. The patients with OH were older than those with negative HUT results, complicating direct comparisons between these groups. Moreover, it is known that both polypharmacy and multimorbidity increase with age (137). In paper 4, we did not adjust for these factors, which could affect both the SctO2 levels and the prevalence of syncope and orthostatic intolerance. Future studies should explore the impact of polypharmacy and multimorbidity on these outcomes.

Paper 5: As already mentioned in the discussion, the risk of dropouts may be a potential limitation of our study. Also, as mentioned previously, we do not include a wash-out period. Our hypothesis is that the beneficial effects of the exercise training program will persist even after the program has ended. While methodological issues have been considered in the discussion section, unforeseen challenges may arise during implementation of the study, which we cannot fully anticipate at this stage.

Future perspectives

The three main concepts of this thesis are related to cerebral oximetry, auto-antibodies and new potential therapies in CVAD. Based on the findings from this thesis a number of future perspectives can be applied.

Cerebral oximetry, besides its role in anaesthesiology, is being increasingly used in experimental. However, it has yet to become an established method in the clinical diagnostic work-up and management of cardiovascular autonomic dysfunction.

Cerebral oximetry may help us understand the mechanisms underlying syncope and orthostatic intolerance, including the relationship between hemodynamic changes and cerebral circulation. It can complement current methods of assessing CBF and improve monitoring of the coupling between hemodynamic changes and symptoms in both VVS and complex syndromes of orthostatic intolerance, such as POTS (60). Additionally, monitoring SctO2 during HUT could provide predictive information for impending VVS, potentially avoiding the need to induce syncope (65).

While individual thresholds for when low cerebral tissue oxygenation causes symptoms likely exist, making cerebral oximetry useful for targeting preventive therapy remains speculative at this stage.

Cognitive dysfunction is common in patients with HF and OH (82, 101), highlighting the importance of monitoring cognitive function in these individuals, perhaps especially among older patients. This may potentially be achieved by measuring SctO2 with NIRS, offering a non-invasive tool to assess cerebral function and guide therapeutic strategies. Exploring the relationship between amnesia for syncope episodes and SctO2 could provide further insights into the cognitive aspects of syncope and the role of cerebral hypoperfusion.

In general, future studies of cerebral oximetry should primarily aim at meticulously measuring SctO2 in parallel with CBF, hemodynamic markers and symptoms during orthostatic provocation in larger patient series. If such studies reveal clinical meaningful alterations in SctO2 in conjunction with symptoms, additional studies investigating if interventions that increase SctO2 may also relieve symptoms of orthostatic intolerance and vice versa, may be the next step.

In addition to cerebral oximetry, the role of autoantibodies in POTS may provide important insights into its pathophysiology. Future studies should explore the mechanisms behind altered autoantibody activity, their downstream effects, and their contribution to the development of POTS. As discussed previously, a recent study, conducted in collaboration with our research team, found no difference in autoantibody concentrations between POTS patients and healthy controls (104). However, examining the function of these antibodies, rather than just their concentrations, could be a valuable area for future research. Trying to identify the parts of antibodies that activate GPCR in POTS may be one such study aim.

The findings from this thesis suggest that POTS may be an autoimmune disorder, highlighting the need for exploring the potential benefit for targeted immunological therapies. However, more systematic research, including randomized placebo-controlled trials, are needed to confirm the role of immunomodulatory therapy in treating POTS. So far, a small controlled randomized trial has not demonstrated a clear benefit of this kind of therapy in POTS (115). It remains to be studied if specific subgroups of POTS patients, such as those with (other) autoimmune manifestations, may benefit from such therapies.

The potential of exercise training as a general complementary treatment for POTS is highly promising. Exercise training has the potential to improve cardiovascular fitness, counter deconditioning, enhance autonomic regulation, and increase overall quality of life for POTS patients (27). Still, exercise training comes with a number of challenges, including both patient specific aspects such as motivation as well as system aspects, such as optimal resource allocation for implementing the training in an economically strained health care system. Future research should focus on optimizing exercise protocols, determining the most effective types and intensities of exercise, and understanding the long-term benefits. Personalized exercise programs tailored to and easy to implement for individual patients needs may also be a key area of development.

Conclusions

This PhD project explores different novel diagnostic methods and treatment options that may add important value for the evaluation, understanding and management of cardiovascular autonomic dysfunction.

- ✓ Application of cerebral oximetry during head up tilt test suggests that POTS patients have lower cerebral tissue oxygenation during orthostatic provocation compared with patients with normal hemodynamic response. However, the decrease in cerebral tissue oxygenation only weakly correlates with HR increase and is not predictive of vasovagal reflex during tilt testing.
- ✓ Older patients with vasovagal syncope and orthostatic hypotension have lower cerebral tissue oxygenation prior to syncope compared with younger patients during orthostatic provocation, independently of concurrent systolic blood pressure levels.
- ✓ Cerebral tissue oxygenation is altered in heart failure. The role of altered SctO2 in heart failure therapy and cognitive function should be further examined.
- ✓ POTS serum mediated membrane G-protein coupled receptor activity has a high predictive value for POTS, providing new insights in the pathophysiology of POTS prompting further research on a possible autoimmune involvement in POTS.
- ✓ Future studies are needed to fully understand these complex disorders and to explore whether interventions that aim to increase cerebral tissue oxygenation and/ or reduce serum mediated G-protein receptor activity may relieve symptoms and prevent long term complications among patients with autonomic dysfunction.
- ✓ Exercise training is increasingly recognized as a valuable component of treatment for patients with POTS. Our cross-over study on exercise training for POTS may provide important information on potential benefits and how it can be implemented in clinical practice for POTS patients.

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