University of Groningen

An investigation of the leading theories behind glaucoma: a case for looking beyond the eye

Loiselle, Allison

DOI:
[10.33612/diss.168309132](https://doi.org/10.33612/diss.168309132)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Loiselle, A. (2021). *An investigation of the leading theories behind glaucoma: a case for looking beyond the eye*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.
<https://doi.org/10.33612/diss.168309132>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

**AN INVESTIGATION OF THE LEADING THEORIES
BEHIND GLAUCOMA: A CASE FOR LOOKING
BEYOND THE EYE**

ALLISON R. LOISELLE

Cover illustration: Allison R. Loiselle

Lay-out and design: Daniëlle Balk, www.persoonlijkproefschrift.nl

Printing: Gildeprint Enschede, gildeprint.nl

© Allison R. Loiselle, 2021

All rights are reserved. No part of this thesis may be reproduced, distributed, stored in a retrieval system, or transmitted in any form or by any means, without prior written permission of the author.

university of
 groningen

An investigation of the leading theories behind glaucoma:
a case for looking beyond the eye

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
maandag 10 mei 2021 om 14:30 uur

Door

Allison Renee Loiselle

geboren op 12 April 1991
te Rhode Island, VS

Promotores

Prof. dr. N.M. Jansonius

Prof. dr. P. van Dijk

Co-promotor

Dr. ir. E. de Kleine

Beoordelingscommissie

Prof. dr. P. Avan

Prof. dr. J. van der Naalt

Prof. dr. M. Hoffmann

TABLE OF CONTENTS

Chapter 1 (*Page 9*)

Introduction and outline

Chapter 2 (*Page 21*)

Noninvasive intracranial pressure assessment using otoacoustic emissions: an application in glaucoma

Chapter 3 (*Page 37*)

Is acetazolamide an effective treatment for glaucoma? Effects on intraocular and intracranial pressure and trans-lamina cribrosa pressure difference: A review

Chapter 4 (*Page 47*)

Intraocular and intracranial pressure in glaucoma patients taking acetazolamide

Chapter 5 (*Page 63*)

Associations between tinnitus and glaucoma suggest a common mechanism: a clinical and population-based study

Chapter 6 (*Page 77*)

Biomarkers in glaucoma and tinnitus suggest low nitric oxide bioavailability and excitotoxicity

Chapter 7 (*Page 91*)

Association of systemic medication exposure with glaucoma progression and glaucoma suspect conversion in the Groningen Longitudinal Glaucoma Study

Chapter 8 (*Page 111*)

Summary and general discussion

Appendices (*Page 121*)

Nederlandse samenvatting

Acknowledgments

“I don’t have many opinions to start with. I have one opinion — one should evaluate things — which is strongly held. I’m never unhappy with the results. I haven’t yet seen a result I didn’t like.”

-Dr. Esther Duflo

1

INTRODUCTION AND OUTLINE

1.1 GLAUCOMA

Glaucoma is a collection of eye diseases with a common endpoint. In the context of this thesis, the focus will be on what is known as primary open angle glaucoma, or POAG. POAG is a chronic and progressive disease that constitutes both structural changes in the eye, which include a thinning retina and damaged optic nerve, and actual functional loss in vision, which can be seen in a visual field test. It is normally associated with a high pressure in the eye, although not all patients have this.⁽¹⁾ “Primary” means that there was no physical trauma or other underlying cause for the disease, and “open angle” means that there is no obstruction in the pathway through which fluid normally flows out of the eye (see Figure 1.1).

FIG 1.1 *“Open angle” glaucoma in which the outflow pathway for the fluid in the eye is unobstructed*

At the beginning of most research articles about glaucoma — including those found in this thesis — you will find some iteration of the sentence “Glaucoma is the second most common cause of blindness worldwide” or “Glaucoma is the leading cause of irreversible blindness”. For researchers in ophthalmology, desensitization to the real impact of these statements has made it easy to hastily read past these introductions to get to the minutiae of the science. Therefore, for those readers who are unfamiliar with the disease and — perhaps more importantly — for those who have spent years researching it, I think it is useful to take a moment to consider the above statements in more detail.

In reference to the first statement: “Glaucoma is the second most common cause of blindness worldwide”, glaucoma is second only to cataracts. Worldwide, 53 million people have POAG (just one subtype of glaucoma) and this number is estimated to rise to 80 million by 2040. This is, in part, due to an increasing population, especially an increase in older people. It may also be due to an increase in urbanization, as those living in urban

areas are 58% more likely to have POAG than those in rural areas.(2) Besides the obvious psychological and emotional burden that glaucoma places on those with the disease and their families, there is a financial burden as well. In terms of provisions of benefits, loss of tax revenue, and direct health care costs, the world is spending an estimated 3.5 trillion US dollars (in 2008 USD) every year on visual impairment.(3) Individually, the U.S and Australia are spending approximately \$3 billion and \$145 million, respectively, on glaucoma. It has also been found that the cost increases with the severity of the disease.(4,5)

This leads us to the second statement: “Glaucoma is the leading cause of *irreversible* blindness”. Unlike cataracts, which can be easily removed in those areas of the world where appropriate health care is available, the visual damage caused by glaucoma cannot be recovered. In addition, the diagnosis itself can often be delayed because it can take a long time before patients experience any noticeable changes in their vision. Unfortunately, it is also possible that some patients will continue to progress even after treatment has commenced. Despite the fact that not all glaucoma patients present with high eye pressure, currently the only available treatment is to lower eye pressure. This can be done with surgery, eye drops, or sometimes oral medications.(6) So, while the global prevalence of all eye diseases rises dramatically, at the very least treatment for cataracts is well understood and we have created scalable treatment options. Yet the rates of glaucoma will simultaneously increase, despite our lack of a complete understanding of its pathophysiological origins.

There are, however, two leading theories for the cause of glaucoma — the mechanical and vascular theories — which will be explored in detail in the following chapters. It is important to note that the aim of this thesis is not to encourage the reader to choose one theory and staunchly support it. It is my hope that readers from all disciplines are able to connect different aspects of this work and disprove others in an attempt to move closer to the truth. By focusing too much on one theory or one discipline we miss opportunities for multidisciplinary collaborations that could more likely solve whole-body problems. This thesis confronts glaucoma as a whole-body disease, and explores not only the eye, but the ear, the brain, the blood, and the effect of medications. It is the result of collaborations with ophthalmologists, audiologists, neurologists, geneticists, and molecular biologists.

1.2 UNDERSTANDING THE MECHANICAL THEORY

The basis of the mechanical theory of glaucoma is that there is an optimal pressure difference between the eye and what is called the “retrobulbar compartment” — or the space behind the eye that is connected to the brain (Figure 1.2). These pressures act from opposing sides on a thin layer, the lamina cribrosa, that supports the axons of the nerve cells required for vision. When this pressure difference is in balance, vision is maintained. However, in the case of glaucoma, this pressure difference is altered and causes shear stress on the nerve cells, leading to subsequent vision loss.(7,8) This pressure relationship could be altered if the patient has an eye pressure that is too high, but also if they have *low* pressure in the retrobulbar compartment (see Figure 1.2). I will subsequently call this latter pressure the

“intracranial pressure” or “ICP”, which despite not specifically referencing the pressure right behind the eye, is used synonymously in mechanical theory research. As proof of the mechanical theory, research has shown that patients with glaucoma have a lower ICP than the healthy population.(9–11) However, other studies investigating ICP in glaucoma patients did not see a difference.(12,13)

FIG 1.2 One possible cause for glaucoma may be from stress on the nerves at the back of the eye caused by a sub-optimal pressure difference between the inside of the eye and behind it.

In order to further elucidate this relationship between ICP and glaucoma, it is necessary to establish a practical and accurate noninvasive method of measuring it. Unlike the eye, which has an external structure by which to measure internal pressure, the brain is enclosed within the skull and has no easily accessible place to measure the pressure. Lumbar punctures, in which a needle is inserted into the spinal canal, are the current gold standard for ICP measurement. But this process is cumbersome, painful for the patient, and can only give information about ICP in the lateral decubitus position (lying down on one side), which may not reflect the pressure in the retrobulbar space. Intraventricular catheters, or pressure devices placed in the brain after removing a part of the skull are also accurate, but for obvious reasons are of no use in medical research for glaucoma. There is one pathway, however, that seems to hold some promise for noninvasive ICP measurement, and that is the connection between the brain and the ear.

The inner ear has ducts that connect to the brain, and therefore the pressure of the fluid within the ear is affected by the ICP. Distortion product otoacoustic emissions (DPOAEs) are an example of utilizing this characteristic of the ear to understand ICP. DPOAEs are a standard ear measurement in which a probe is placed in the ear canal and two tones are played simultaneously which evoke subsequent vibrations in a membrane in a part of the ear called the cochlea. The magnitude and phase of these vibrations are dependent on the

cochlear pressure, and therefore the ICP. Previous studies have shown that DPOAEs can accurately measure changes in ICP.(14–18) One limitation of DPOAEs is that, currently, they can only measure *changes* in pressure and not absolute pressure. Therefore DPOAEs can be used when altering ICP by changing a subject's body position, or before and after taking a drug that lowers or increases ICP.

Chapters 2, 3, and 4 will focus primarily on the mechanical theory of glaucoma. The aim of chapter 2 was to determine the exact relationship between DPOAEs and body position and to see if this relationship was different between patients with glaucoma and healthy subjects. Chapter 3 and 4 focus on a glaucoma drug that lowers eye pressure but is also known to affect ICP, called acetazolamide. The aim of these chapters was to determine to what extent this drug affects both eye pressure and ICP in glaucoma patients and to explore the potential negative effects of alterations in the two pressures.

1.3 UNDERSTANDING THE VASCULAR THEORY

In the vascular theory of glaucoma, it is posited that the characteristic optic nerve damage, retinal thinning and subsequent vision loss is a consequence of insufficient blood flow to the eye.(19,20) When the nerves at the back of the eye are not getting sufficient blood flow, this means they are also not receiving enough oxygen, and the cells can die. This suboptimal blood flow can be a result of a multitude of risk factors that have been associated with glaucoma including, but not limited to: changes in blood pressure during the day and night, age, high and low blood pressure, and cardiovascular disease.(21–23)

It is also possible that, for some patients, glaucoma is just one external manifestation of a wider problem of vascular dysregulation throughout the whole body.(24,25) This means that, for some reason, blood flow is not changing to meet the demands of specific tissues in the body. If this is true, it is possible that organs other than the eye could be affected by insufficient blood flow in patients with glaucoma. After some anecdotal evidence and complaints about tinnitus from glaucoma patients in our lab, we decided to explore if this insufficient blood flow theory applied to the ear as well. Tinnitus affects 10-15% of the adult population but is still a poorly understood disease that is often associated with hearing loss and aging. It is normally characterized by a ringing or rushing sound in the ears and can be subjective (only perceived by the patient) or, more seldomly, objective (also perceivable by an observer).(26,27) Only one previous study had demonstrated a relationship between glaucoma and tinnitus, but this was only in glaucoma patients with a normal eye pressure.(28) In chapter 5, we confirm this finding in POAG patients in our lab and in a large population-based study and show that glaucoma patients are more likely to have tinnitus than those without glaucoma, even after controlling for various influential factors like age, gender, and socioeconomic status.(29) We hypothesize that the connection between the two diseases may be an impairment in the production of a molecule that is crucial for the vascular system, called nitric oxide.

Nitric oxide (NO) is a small gas molecule made up of one nitrogen and one oxygen atom. The endothelium (or inner lining) of the vascular system uses NO to relax the surrounding

smooth muscle and therefore cause the vessels to dilate. This process is called vasodilation and it is critical for increasing blood flow. In a normal healthy person, the main way that NO is produced is inside the body, where a specific enzyme helps to convert one amino acid to NO and a different amino acid. When this process is impaired, NO (and subsequently blood flow) can be reduced.(30) To make matters worse, the impairment of this process yields a byproduct that can cause even more damage: free radicals. A free radical called peroxynitrite can be formed when NO is not being produced properly. These molecules have one unpaired electron, which means that they are very unstable and can react quickly, taking electrons from whichever molecules are closest (a process called oxidation). This can lead to a chain reaction and cause a certain type of damage to cells that is called “oxidative stress”. So the result is compounded, not only is blood flow decreased (and therefore the amount of oxygen that can get to the eye or the ear), but there is an increase in oxidative stress which can also kill nerve cells that are necessary for vision and hearing. Research has already shown that markers of NO are decreased in patients with glaucoma and patients with tinnitus and that they even have genetic changes linked to the production of NO.(31–36) In chapter 6, we evaluate blood samples from glaucoma patients and controls with and without tinnitus.

Lastly, as vascular insufficiency may be related to glaucoma, it is important to look at the impact of systemic medications (medications that can affect the whole body) that are linked to the vascular system. Glaucoma is a disease typically found in older people, the same age range in which many people begin taking drugs for high cholesterol (statins) and high blood pressure (antihypertensives). Statins have been shown in some studies to have a protective effect for glaucoma that may not be related to lowering of cholesterol.(37–39) Antihypertensives have been shown to be both helpful and harmful for glaucoma.(40–44) These studies are often difficult to interpret all together because there are a multitude of factors that can be slightly different in each article. These include: the age distribution of the study population, the strictness of the definition used for glaucoma, whether or not they are investigating the progression of the disease or its onset, how long they follow patients for, and the specific class of antihypertensive used. To make things more confusing, over-treating for hypertension can lead to blood pressure that is too low, which has also been shown to be a risk factor for glaucoma.(20,45) Taking all of these factors into account is critical for a complete understanding of how these commonly prescribed drugs may influence those with, or at risk for glaucoma.

Chapters 5, 6, and 7 will focus primarily on the vascular theory of glaucoma. The aim of chapter 5 was to determine if there is a relationship between glaucoma and tinnitus and chapter 6 was a follow-up on the proposed mechanism behind this relationship, namely impaired nitric oxide production. Finally, chapter 7 aimed to look at the effect of systemic medications on both the conversion of glaucoma suspects to a glaucoma diagnosis and also the progression of the disease.

REFERENCE

1. Casson RJ, Chidlow G, Wood JPM, Crowston JG, Goldberg I. Definition of glaucoma: clinical and experimental concepts [Internet]. Vol. 40, Clinical & Experimental Ophthalmology. 2012. p. 341–9. Available from: <http://dx.doi.org/10.1111/j.1442-9071.2012.02773.x>
2. Tham Y-C, Li X, Wong TY, Quigley HA, Aung T, Cheng C-Y. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*. 2014 Nov;121(11):2081–90.
3. Gordoio A, Cutler H, Pezzullo L, Gordon K, Cruess A, Winyard S, et al. An estimation of the worldwide economic and health burden of visual impairment. *Glob Public Health*. 2012;7(5):465–81.
4. Varma R, Lee PP, Goldberg I, Kotak S. An Assessment of the Health and Economic Burdens of Glaucoma [Internet]. Vol. 152, American Journal of Ophthalmology. 2011. p. 515–22. Available from: <http://dx.doi.org/10.1016/j.ajo.2011.06.004>
5. Traverso CE, Walt JG, Kelly SP, Hommer AH, Bron AM, Denis P, et al. Direct costs of glaucoma and severity of the disease: a multinational long term study of resource utilisation in Europe. *Br J Ophthalmol*. 2005 Oct;89(10):1245–9.
6. Lee DA, Higginbotham EJ. Glaucoma and its treatment: A review [Internet]. Vol. 62, American Journal of Health-System Pharmacy. 2005. p. 691–9. Available from: <http://dx.doi.org/10.1093/ajhp/62.7.691>
7. Morgan WH, Yu DY, Cooper RL, Alder VA, Cringle SJ, Constable IJ. The influence of cerebrospinal fluid pressure on the lamina cribrosa tissue pressure gradient. *Invest Ophthalmol Vis Sci*. 1995 May;36(6):1163–72.
8. Jonas JB, Ritch R, Panda-Jonas S. Cerebrospinal fluid pressure in the pathogenesis of glaucoma. *Prog Brain Res*. 2015 Sep 9;221:33–47.
9. Berdahl JP, Fautsch MP, Stinnett SS, Allingham RR. Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. *Invest Ophthalmol Vis Sci*. 2008 Dec;49(12):5412–8.
10. Berdahl JP, Rand Allingham R, Johnson DH. Cerebrospinal Fluid Pressure Is Decreased in Primary Open-angle Glaucoma [Internet]. Vol. 115, Ophthalmology. 2008. p. 763–8. Available from: <http://dx.doi.org/10.1016/j.ophtha.2008.01.013>
11. Ren R, Jonas JB, Tian G, Zhen Y, Ma K, Li S, et al. Cerebrospinal Fluid Pressure in Glaucoma [Internet]. Vol. 117, Ophthalmology. 2010. p. 259–66. Available from: <http://dx.doi.org/10.1016/j.ophtha.2009.06.058>
12. Lindén C, Qvarlander S, Jóhannesson G, Johansson E, Östlund F, Malm J, et al. Normal-Tension Glaucoma Has Normal Intracranial Pressure: A Prospective Study of Intracranial Pressure and Intraocular Pressure in Different Body Positions. *Ophthalmology*. 2018 Mar;125(3):361–8.
13. Pircher A, Remonda L, Weinreb RN, Killer HE. Translaminar pressure in Caucasian normal tension glaucoma patients. *Acta Ophthalmol*. 2017 Nov;95(7):e524–31.
14. Avan P, Giraudet F, Chauveau B, Gilain L, Mom T. Unstable distortion-product otoacoustic emission phase in Menière's disease. *Hear Res*. 2011 Jul;277(1-2):88–95.
15. Büki B, Chomici A, Dordain M, Lemaire JJ, Wit HP, Chazal J, et al. Middle-ear influence on otoacoustic emissions. II: contributions of

-
- posture and intracranial pressure. *Hear Res.* 2000 Feb;140(1-2):202–11.
16. Bershad EM, Urfy MZ, Pechacek A, McGrath M, Calvillo E, Horton NJ, et al. Intracranial pressure modulates distortion product otoacoustic emissions: a proof-of-principle study. *Neurosurgery.* 2014 Oct;75(4):445–54; discussion 454–5.
 17. Voss SE, Adegoke MF, Horton NJ, Sheth KN, Rosand J, Shera CA. Posture systematically alters ear-canal reflectance and DPOAE properties [Internet]. Vol. 263, *Hearing Research.* 2010. p. 43–51. Available from: <http://dx.doi.org/10.1016/j.heares.2010.03.003>
 18. Williams MA, Malm J, Eklund A, Horton NJ, Voss SE. Distortion Product Otoacoustic Emissions and Intracranial Pressure During CSF Infusion Testing. *Aerosp Med Hum Perform.* 2016;87(10):844–51.
 19. Leske MC. Ocular perfusion pressure and glaucoma: clinical trial and epidemiologic findings. *Curr Opin Ophthalmol.* 2009 Mar;20(2):73–8.
 20. Topouzis F. The role of ocular perfusion pressure in glaucoma [Internet]. Vol. 92, *Acta Ophthalmologica.* 2014. p. 0–0. Available from: <http://dx.doi.org/10.1111/j.1755-3768.2014.2221.x>
 21. Hayreh SS. Blood flow in the optic nerve head and factors that may influence it. *Prog Retin Eye Res.* 2001 Sep;20(5):595–624.
 22. Tielsch JM. Hypertension, Perfusion Pressure, and Primary Open-angle Glaucoma [Internet]. Vol. 113, *Archives of Ophthalmology.* 1995. p. 216. Available from: <http://dx.doi.org/10.1001/archophth.1995.01100020100038>
 23. Leske MC, Cristina Leske M. Risk Factors for Open-angle Glaucoma: The Barbados Eye Study-Reply [Internet]. Vol. 114, *Archives of Ophthalmology.* 1996. p. 235. Available from: <http://dx.doi.org/10.1001/archophth.1996.01100130229030>
 24. Harris A, Siesky B, Wirostko B. Cerebral blood flow in glaucoma patients. *J Glaucoma.* 2013 Jun;22 Suppl 5:S46–8.
 25. Pasquale LR. Vascular and autonomic dysregulation in primary open-angle glaucoma. *Curr Opin Ophthalmol.* 2016 Mar;27(2):94–101.
 26. Baguley D, McFerran D, Hall D. Tinnitus. *Lancet.* 2013 Nov 9;382(9904):1600–7.
 27. Lockwood AH, Salvi RJ, Burkard RF. Tinnitus. *N Engl J Med.* 2002 Sep 19;347(12):904–10.
 28. Konieczka K, Choi HJ, Koch S, Fankhauser F, Schoetzau A, Kim DM. Relationship between normal tension glaucoma and Flammer syndrome. *EPMA J.* 2017 Jun;8(2):111–7.
 29. Loiselle AR, Neustaeter A, de Kleine E, van Dijk P, Jansonius NM. Associations between tinnitus and glaucoma suggest a common mechanism: A clinical and population-based study. *Hear Res.* 2020 Feb;386:107862.
 30. Bryan NS, Loscalzo J. Nitrite and Nitrate in Human Health and Disease. *Humana Press*; 2017. 349 p.
 31. Galassi F, Renieri G, Sodi A, Ucci F, Vannozzi L, Masini E. Nitric oxide proxies and ocular perfusion pressure in primary open angle glaucoma. *Br J Ophthalmol.* 2004 Jun;88(6):757–60.
 32. Huang W, Wang W, Zhou M, Zhang X. Association of single-nucleotide polymorphism rs4236601 near caveolin 1 and 2 with primary open-angle glaucoma: a meta-analysis. *Clin Experiment Ophthalmol.* 2014 Aug;42(6):515–21.
 33. Kang JH, Wiggs JL, Rosner BA, Hankinson SE, Abdrabou W, Fan BJ, et
-

- al. Endothelial nitric oxide synthase gene variants and primary open-angle glaucoma: interactions with sex and postmenopausal hormone use. *Invest Ophthalmol Vis Sci*. 2010 Feb;51(2):971–9.
34. Emam WA, Zidan HE, Abdulhalim B-EH, Dabour SA, Ghali MA, Kamal AT. Endothelial nitric oxide synthase polymorphisms and susceptibility to high-tension primary open-angle glaucoma in an Egyptian cohort. *Mol Vis*. 2014 Jun 12;20:804–11.
35. Teranishi M, Uchida Y, Nishio N, Kato K, Otake H, Yoshida T, et al. Polymorphisms in genes involved in the free-radical process in patients with sudden sensorineural hearing loss and Ménière's disease. *Free Radic Res*. 2013 Jul;47(6-7):498–506.
36. Neri S, Signorelli S, Pulvirenti D, Mauceri B, Cilio D, Bordonaro F, et al. Oxidative stress, nitric oxide, endothelial dysfunction and tinnitus. *Free Radic Res*. 2006 Jun;40(6):615–8.
37. Whigham B, Oddone EZ, Woolson S, Coffman C, Allingham RR, Shieh C, et al. The influence of oral statin medications on progression of glaucomatous visual field loss: A propensity score analysis. *Ophthalmic Epidemiol*. 2018 Jun;25(3):207–14.
38. Stein JD, Newman-Casey PA, Talwar N, Nan B, Richards JE, Musch DC. The Relationship Between Statin Use and Open-Angle Glaucoma [Internet]. Vol. 119, *Ophthalmology*. 2012. p. 2074–81. Available from: <http://dx.doi.org/10.1016/j.ophtha.2012.04.029>
39. Leung DY, Li FCH, Kwong YYY, Tham CCY, Chi SCC, Lam DSC. Simvastatin and disease stabilization in normal tension glaucoma: a cohort study. *Ophthalmology*. 2010 Mar;117(3):471–6.
40. Horwitz A, Klemp M, Jeppesen J, Tsai JC, Torp-Pedersen C, Kolko M. Antihypertensive Medication Postpones the Onset of Glaucoma: Evidence From a Nationwide Study. *Hypertension*. 2017 Feb;69(2):202–10.
41. Iskedjian M, Walker JH, Desjardins O, Robin AL, Covert DW, Bergamini MVW, et al. Effect of selected antihypertensives, antidiabetics, statins and diuretics on adjunctive medical treatment of glaucoma: a population based study. *Curr Med Res Opin*. 2009 Aug;25(8):1879–88.
42. Zheng W, Dryja TP, Wei Z, Song D, Tian H, Kahler KH, et al. Systemic Medication Associations with Presumed Advanced or Uncontrolled Primary Open-Angle Glaucoma. *Ophthalmology*. 2018 Jul;125(7):984–93.
43. Müskens RPHM, de Voogd S, Wolfs RCW, Witterman JCM, Hofman A, de Jong PTVM, et al. Systemic antihypertensive medication and incident open-angle glaucoma. *Ophthalmology*. 2007 Dec;114(12):2221–6.
44. Owen CG, Carey IM, Shah S, de Wilde S, Wormald R, Whincup PH, et al. Hypotensive medication, statins, and the risk of glaucoma. *Invest Ophthalmol Vis Sci*. 2010 Jul;51(7):3524–30.
45. Bowe A, Grünig M, Schubert J, Demir M, Hoffmann V, Kütting F, et al. Circadian Variation in Arterial Blood Pressure and Glaucomatous Optic Neuropathy--A Systematic Review and Meta-Analysis. *Am J Hypertens*. 2015 Sep;28(9):1077–82.

MECHANICAL THEORY

❧ 2 ❧

Noninvasive intracranial pressure assessment using otoacoustic emissions: an application in glaucoma

Allison R. Loiselle, Emile de Kleine, Pim van Dijk, Nomdo M. Jansonius

(Published: PLoS ONE. October 2018; 13(10):e0204939)

ABSTRACT

The theory that glaucoma patients have a lower intracranial pressure (ICP) than healthy subjects is a controversial one. The aim of this study was to assess ICP noninvasively by determining the relationship between distortion product otoacoustic emission (DPOAE) phase and body position and to compare this relationship between patients with primary open angle glaucoma (POAG), patients with normal tension glaucoma (NTG), and controls. The relationship was also calibrated using published data regarding invasive measurements of ICP versus body position. DPOAEs were measured in 30 controls and 32 glaucoma patients (17 POAG, 15 NTG) at the following body positions (assuming 90° as upright): 45, 30, 20, 10, 0 (supine), -10, and -20°. DPOAE phase had a clear, nonlinear relationship with body position. The mean DPOAE phase shifts between the two most extreme body positions (45 to -20°) were 73.6, 80.7, and 66.3° for healthy, POAG, and NTG, respectively ($P=0.73$), and the groups showed the same, nonlinear behaviour. This indicates that there is no evidence that glaucoma patients have a reduced ICP. When calibrated with invasive data, ICP and DPOAE phase were linearly related over an ICP of 3 mmHg. This suggests that, more broadly, DPOAEs could be used in the future to monitor changes in ICP in a clinical setting and to measure dynamic changes in ICP such as diurnal fluctuations or changes induced by certain medications.

2.1 INTRODUCTION

Primary open angle glaucoma (POAG) is a chronic and progressive eye disease characterized by loss of retinal ganglion cells, thinning of the retinal nerve fiber layer, and subsequent visual field loss. If left untreated, it can eventually lead to blindness. Previously, an elevated intraocular pressure (IOP) was deemed to be the key factor in the pathophysiology of glaucoma. However, there is a variant of POAG in which the patients have a normal IOP (normal tension glaucoma [NTG])(1). One potential explanation for this is that the relationship between IOP and intracranial pressure (ICP) is the key factor, rather than IOP itself (2,3).

Alterations in IOP, ICP, or both can lead to a change in the pressure gradient across the lamina cribrosa (LC) — known as the trans lamina cribrosa pressure difference (TLCPD) — and cause it to bulge, therefore damaging the nerve fibers. It has been shown with lumbar punctures (LPs) that patients with glaucoma have a lower ICP than the healthy population and that those with NTG have the lowest (4–6). As further evidence, patients with normal pressure hydrocephalus who received shunts, which can significantly lower ICP, had a 40 fold increase in the rate of NTG when compared with the general population (7). There is, however, controversy over this theory (8).

LPs are invasive and thus limit a researcher’s ability to conduct experiments with a sufficient number of subjects. In addition, LP opening pressures are only a proxy of the actual ICP as they are measured at the lower part of the spine and typically only in the lateral decubitus position. They are especially not likely to be representative of the pressure behind the LC. This is important because it is possible that NTG is related to an inability to maintain a certain pressure in the upright position, rather than the lateral decubitus position. Interestingly, a recent study by Linden et al. (8) aimed to use LPs to estimate ICP at the LC as accurately as possible by accounting for the hydrostatic gradients between the auditory meatus and the LC. LPs were done and ICP was measured continuously using a pressure transducer in various body positions but no differences were found in ICP between a small group of NTG patients and controls. A noninvasive device that measures the pressure at the level of the brain rather than in the lower part of the spinal canal could potentially further this field tremendously.

Otoacoustic emissions are sounds that originate in the cochlea and can be easily used to measure cochlear function noninvasively (9,10). One particular type of emission is the distortion product otoacoustic emission (DPOAE), which is emitted by the inner ear in response to two tones at specified levels and frequencies. These emissions are thought to depend on ICP because there is a connection between the cranium and inner ear via the cochlear and endolymphatic aqueducts. When ICP fluctuates, the pressure on the stapes also changes, affecting the transmission of sound in the middle ear. Previous research has shown that DPOAE phase shifts can accurately represent changes in ICP (11–14). As such, a DPOAE measurement potentially contains all the desired properties of an ideal ICP measurement. One limitation is that the recorded DPOAE phase has an unknown, subject-specific offset. As a result, if the relationship between ICP and the DPOAE phase would be linear, changes in phase would only convey changes in pressure. In case of a nonlinear relationship (e.g. the phase changes only for pressures above or below a certain value),

however, an absolute pressure measurement should be feasible as well. In this study we explore the clinical value of the DPOAE phase shift for ICP assessment and apply the technique to glaucoma patients.

The aim of this study was to (1) determine the relationship between DPOAEs and body position and to (2) compare this relationship between POAG, NTG, and controls. Finally, we aimed to (3) calibrate this relationship using published data regarding LP-based measurements of ICP versus body position.

2.2 METHODS

2.2.1 Study population

Subjects with healthy eyes who responded to our advertisement and glaucoma patients who were selected from the Groningen Longitudinal Glaucoma Study database (15), received an information letter and informed consent form. The ethics board of the University Medical Center Groningen (UMCG) approved the study protocol. All participants provided written informed consent. The study followed the tenets of the Declaration of Helsinki.

In order to be eligible to participate in this study, subjects in all groups had to meet the following inclusion criteria: 50 to 70 years of age and presence of detectable DPOAEs in at least one ear. Additionally, for controls: IOP of 21 mmHg or lower, no eye disease, and no family history of glaucoma (determined by a questionnaire). To exclude eye disease, we performed optical coherence tomography (OCT-HS100; Canon, Tokyo, Japan; considered normal if the mean retinal nerve fiber layer and retinal ganglion cell layer thickness in macular area were above the 5th percentile), frequency doubling technology (FDT; Carl Zeiss, Jena, Germany; no reproducibly abnormal test locations allowed in C20-1 screening mode), and a measurement of visual acuity (visual acuity at least 0.8 in both eyes). For POAG: diagnosed glaucoma and IOP over 21 mmHg before the onset of IOP lowering treatment. For NTG: diagnosed glaucoma and IOP of 21 mmHg or lower before the onset of IOP lowering treatment and at any time during follow-up. Glaucoma was defined according to Heeg et al (15). We required a reproducible (same hemifield and at least partially overlapping) visual field defect (Humphrey Field Analyzer 30-2 SITA fast; Carl Zeiss, Jena, Germany; criterion: ‘glaucoma hemifield test’ outside normal limits) in at least one eye that had to be compatible with glaucoma and without any other explanation. Subjects taking acetazolamide were excluded in this study as this drug has been shown to change ICP (16–18).

2.2.2 DPOAE parameters

DPOAEs were measured using hardware (Elios) and software (Echosoftware version 2.4.2) developed by Echodia (St. Beauzire, France). To ensure the highest magnitude responses at the $2f_1-f_2$ emission, a fixed rate of $f_2/f_1=1.20$ with tones at frequencies $f_1=1000$ Hz and $f_2=1200$ Hz and levels $L_1=L_2=72$ dB SPL were used. All DPOAE measurements were completed in a sound-isolated audiometric room in the otorhinolaryngology clinic.

2.2.3 Measurement Protocol

Blood pressure (Omron Model M6 Comfort, Omron Healthcare Co., Ltd.) and upright IOP (iCare Pro tonometer, Icare Finland Oy) were measured. Subjects were then secured onto the tilt table (Ironman iControl 400 Disk Brake Inversion System, Paradigm Health and Wellness Inc.). Body mass index (BMI) was determined using self-reported height and weight.

DPOAEs were measured at the following body positions (assuming 90° as upright): 45, 30, 20, 10, 0 (supine), -10, and -20°. At each position, 30 seconds were allowed for normalization of the emission, and presumably the ICP (12,19). This was followed by 5 DPOAE measurements that were performed over approximately 20 seconds. IOP was measured again at the supine position. The ear probe was then removed and subjects were allowed a short break before repeating the test. In this way we were able to determine test-retest variability with (between test) and without (within test) replacing the probe.

2.2.4 Data analysis

Groups were described with mean and standard deviation (SD) for normally distributed variables; means were compared using one-way analysis of variance (ANOVA). For variables with a skewed distribution, we used median and interquartile range (IQR) for descriptive statistics and the Kruskal-Wallis test for comparing medians of groups. Proportions were compared using a chi-square test.

The stimulus and data collection protocols are already described in detail by Avan et al (20). At each body position, 5 DPOAE measurements were taken, which took approximately a total of 20 seconds of measurement time. For the within test test-retest variability, the first 2 and last 2 of these 5 measurements were averaged for each body position and compared. For the between test test-retest variability, the average phase over the full 20 seconds was compared for the first and second test, between which the ear probe had been removed and replaced. Test-retest variability was presented as the SD of differences of both measurements.

Due to the subject specific offset of the DPOAEs, normalization of the data was required. For each subject, an average phase over all body positions was calculated and the individuals' data were normalized using this value. Individual measurements with a signal to noise ratio (SNR) of less than 3 dB were excluded. If fewer than 2 measurements with a SNR over 3 dB were available at more than one body position (out of 5 measurements per body position), those subjects were removed entirely. All analyses were performed using R (version 3.0.2; R Foundation for Statistical Computing, Vienna, Austria). A P-value of 0.05 or less was considered statistically significant.

2.3 RESULTS

Of the 35 healthy subjects who agreed to participate, 1 was excluded because of an abnormal eye exam and 1 because of lack of emissions. Of the 43 glaucoma patients who agreed to participate, 7 were excluded because of lack of emissions. Due to signals below the noise floor, we could not analyze the data of 3 of 35 controls, 1 of 22 POAG patients, and 3 of 21 NTG patients. Therefore a total of 62 participants were included in the analysis (30 controls, 17 POAG, and 15 NTG). Table 2.1 shows the demographics and ocular characteristics of the study population. The groups had approximately the same mean age, did not differ regarding gender, and there were no significant differences in any of the other demographic criteria. The visual field defects were similar in both the better eye and the worse eye for POAG and NTG. IOP (reported as the mean of both eyes) increased more from upright to supine for the patient groups than for the controls, although this was only statistically significant between controls and POAG ($P=0.01$).

Table 2.1 | Demographics of the study population ($n=62$; mean $\pm$ SD unless stated otherwise)

Group	Healthy ($n=30$)	POAG ($n=17$)	NTG ($n=15$)	P-value
Gender (% female)	43%	35%	60%	0.36
Age (yrs)	58.4 ± 6.4	61.6 ± 4.1	62.1 ± 4.7	0.05
BMI (kg/m^2)	25.9 ± 3.1	25.7 ± 3.5	24.8 ± 3.9	0.58
SBP (mmHg)	131.4 ± 11.1	139.8 ± 18.9	131.1 ± 14.9	0.13
DBP (mmHg)	83.5 ± 9.6	87.0 ± 9.9	83.2 ± 10.3	0.44
VF MD of better eye (dB; median [IQR])	-	-2.5 (-6.8 to -0.7)	-3.3 (-4.2 to -2.3)	0.56
VF MD of worse eye (dB; median [IQR])	-	-12.3 (-15.5 to -4.9)	-9.8 (-4.7 to -21.8)	0.74
IOP0 (mmHg; median [IQR])	-	30.0 (28.0 to 34.0)	17.5 (15.2 to 19.7)	-
Upright IOP (mmHg; median [IQR])	15.1 (14.2 to 15.8)	15.6 (14.9 to 16.4)	15.4 (12.7 to 16.0)	0.34
Difference in IOP supine to upright (mmHg; median [IQR])	1.5 (0.9 to 2.0)	2.6 (1.9 to 3.4)	1.9 (0.9 to 3.8)	0.01

SD standard deviation, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *VF MD* standard automated perimetry mean deviation, *IOP0* intraocular pressure before onset of IOP lowering treatment.

Figure 2.1 shows the DPOAE phase as a function of body position for the 30 healthy subjects, averaged for the first and second test. There is a clear relationship between phase and body position, especially when tilting to the 10° body position and lower. The relationship is not strictly monotonic, as the phase seems to increase again for the 30° body position and higher. This finding is likely robust, as it could be observed in both the first and the second test if analyzed separately. Initially we also measured -30° below horizontal, but that position had to be abandoned due to complaints and discomfort for the participants. In the 12 healthy subjects, 9 POAG, and 2 NTG patients in whom we measured this position (-30°), we found a clear further increase in phase compared to the -20° position of (mean $\pm$ standard error) 15.4 ± 3.7 , 19.4 ± 9.9 , and $24.6 \pm 14.8^\circ$ for healthy, POAG, and NTG, respectively.

FIG 2.1 Relationship between body position and DPOAE phase (mean $\pm$ standard error) for healthy subjects ($n=80$). Data were averaged over both tests. Body positions are in reference to 90° as upright.

The observed relationship between DPOAE phase and body position was also shown in POAG and NTG (Figure 2.2). The mean overall phase shifts between the two most extreme body positions (45° to -20°) were 73.6, 80.7, and 66.3° for healthy, POAG, and NTG, respectively. Figure 2.3 shows the corresponding scatter plots. One NTG subject was not included in this plot because he/she did not feel comfortable tilting to the furthest position. Despite the trend for a smaller mean overall phase shift in NTG patients, an ANOVA for the overall phase shift revealed that there were no significant differences between the healthy subjects and either of the patient groups ($P=0.73$).

In order to be able to interpret the DPOAE phase data, we merged our data of phase as a function of body position presented in Figure 2.1 with published data regarding ICP as a function of body position, based on LP measurements by Linden et al. (8). Figure 2.4A shows their healthy subject data, adapted by interpolation to our body positions (interpolation was not possible for -20°); Figure 2.4B presents the merged data, showing DPOAE phase as a function of ICP. Above an ICP of approximately 3 mmHg, phase was linearly related to ICP, with a slope of 4 degree/mmHg. Below 3 mmHg, there was no clear relationship between phase and ICP.

FIG 22 Relationship between body position and DPOAE phase (mean $\pm$ standard error) for healthy subjects ($n=30$) and POAG ($n=17$) and NTG ($n=15$) patients. Data were averaged over both tests. Body positions are in reference to 90° as upright.

FIG 23 DPOAE phase shift for healthy subjects ($n=30$) and POAG ($n=17$) and NTG ($n=14$) patients between the body positions of 15° and -20° .

FIG 2.4 DPOAE phase as a function of ICP. (A) ICP as a function of body position for healthy subjects ($n=11$) from Linden et al (8) adapted by interpolation to our body positions. (B) merged data.

As previously mentioned, the ear probe was removed between the first and second test to examine the test-retest variability of the device. Figure 2.5 shows scatter plots of the within test (panel A) and between test (panel B) test-retest variability of absolute phase at supine and also the SD of differences at all body positions (panel C). Nine subjects were removed from the between tests comparison, 6 due to a low SNR (for criteria see Methods section) in one of the tests and 3 NTG subjects that were not able to complete the second test due to nausea (see Discussion section). The smallest between test variability, with a SD of differences of 22.2° , occurred at the 10° body position. The within test variability was low and fairly stable across all body positions, with a SD of differences ranging from 5.3° to 10.2° (corresponding to approximately 2 mmHg of ICP; see Figure 2.4B).

Fig 2.5 (A) Within test ($n=62$) and (B) Between test ($N=53$) test-retest variability, in phase at supine for all subjects and (C) the SD of differences for each body position.

2.4 DISCUSSION

There is a nonlinear relationship between DPOAE phase and body position that is similar in healthy subjects and patients with POAG and NTG. Above an ICP of approximately 3 mmHg, there is a linear relationship between DPOAE phase and ICP with a slope of 4 degree/mmHg; below 3 mmHg, there is no clear relationship between phase and ICP.

Previous studies have demonstrated that DPOAE phase shifts can accurately represent changes in ICP. As one step of a larger experiment, Büki et al. (12) measured DPOAEs in 12 healthy subjects who were tilted on a table between upright and -30° and compared this to data from 5 patients with hydrocephalus and found that “ICP is the key element for all auditory modifications associated with posture”. In another study (13), DPOAEs were measured in 12 healthy subjects tilted from upright to -45° . At the same DPOAE frequency as used in the current study, they demonstrated DPOAE phase shifts of about 54° invoked by posture change. The overall shift found in the current study was of the same order of magnitude ($\sim 70^\circ$ for a body position of -20 to $+45^\circ$). De Kleine et al. (19) provided an equation to calculate changes in ICP from changes in body position. They predicted an ICP change of ~ 10 mmHg for -10 vs 45° . The DPOAE phase shift for this body position change was approximately 50° in our study. As can be seen in Figure 2.4, these data are in agreement with each other. In the first study to directly compare DPOAEs with LPs, Bershad et al. (11,21) found that large changes in ICP (>15 mmHg) between opening and closing pressures were significantly associated with changes in DPOAE phase. In another study (14), DPOAEs were measured in 8 subjects undergoing CSF infusion testing, and it was shown that for ICP changes of ~ 12 mmHg or more over baseline, DPOAE phase changes were significant. In the current study, we have reproduced the finding that changes in DPOAE phase can represent changes in ICP. However, our data suggest that, with appropriate probe placement and sufficient averaging, much smaller changes in ICP could be detected (see below).

For many of the subjects there was a noticeable increase in phase that occurs toward the upright position, so that the minimum phase occurred at 30° and not at 45° (see Figs. 2.1 and 2.2). There are two physiological possibilities for this phenomenon. One is that ICP has its minimum somewhere halfway between supine and upright rather than at upright. In an invasive ICP study (22) it was demonstrated that this occurred in approximately 5% of subjects. In the current study, however, an observable minimum in phase at body positions lower than 45° occurred in over 50% of subjects. Another possibility is that when there is a negative ICP, as is often the case in the upright position, the stapes is pulled inward into the oval window and may elicit a response in DPOAE phase similar to when it is pushed outward. In this case the minimum phase would occur at a body position intermediate between upright and supine, when the stapes is in a neutral position.

There were some limitations in the current study: First, DPOAEs were not measured at the upright position which was due to a limitation of the tilt table. However, within the range of body positions included in this study, the nonlinear relationship between phase and body position could clearly be uncovered. It is also important to note that 3 NTG subjects could not complete the second part of the test due to nausea and dizziness. No other subjects had

any problems with the test. Interestingly, this supports the concept that vascular factors and impairment of ocular blood flow might play a role in the pathogenesis of NTG (23). Indeed 2 out of the 3 subjects had low blood pressure (106/73 mmHg and 107/79 mmHg). Finally, there was a large intersubject variation in the overall DPOAE phase shift. At first sight, this seems to hamper the use of DPOAE phase for ICP assessment. However, it should be realized that ICP itself also shows a significant variability (22,24).

What information regarding ICP can be obtained from the DPOAE measurements? Importantly, due to a subject-specific offset, there is no one-to-one relationship between DPOAE phase and ICP. For an ICP above 3 mmHg, there is a linear relationship between DPOAE phase and ICP, which implies that a change in phase can be converted into a change in ICP, where 4° corresponds to 1 mmHg (Figure 2.4B). This suggests that it is possible to monitor ICP changes in a subject or patient in the supine position, in which ICP should be amply above 3 mmHg (Figure 2.4A). The accuracy (expressed as standard deviation of differences) is approximately 2 mmHg without probe movement, and 5 mmHg with probe movement or replacement. This is already clinically useful; a more stable and reproducible probe positioning could further improve the accuracy. Below 3 mmHg, the phase does not change further, and may even start to change in the opposite direction. This nonlinear behaviour offers potentially an opportunity to obtain information regarding absolute ICP from DPOAE measurements. In the case of a low ICP, one would expect the curve of phase as a function of body position (Figure 2.1) to move leftward, meaning a more extreme tilt position is required to elicit a DPOAE response. Related to that, lower ICP patients would have a smaller overall phase shift when changing body position from upright to head down. On the other hand, in the case of a high ICP, one would expect the curve to move rightward with a larger overall phase shift.

There are many theories as to why glaucoma may occur in patients with a normal eye pressure. One theory is that NTG patients simply have thin corneas, yielding erroneously low IOP measurements. In this study, however, the corneal thickness for POAG and NTG were similar (544 μm and 554 μm , respectively), signifying that another explanation is necessary. What do our results suggest regarding ICP in NTG? The overall phase shift seemed smaller in the NTG patients than in the healthy subjects or POAG patients (Figure 2.3), but the difference was not significant. Also, the phase as a function of body position curve of the NTG patients (Figure 2.2) did not show a clear leftward or rightward shift when compared to that of the healthy subjects or POAG patients. A shift of more than 5° along the body position axis seems unlikely, which suggests that the mean difference in ICP between our NTG patients, healthy subjects, and POAG patients is less than 1-2 mmHg. As such, our results agree with the findings of Linden et al (8), who reported no difference in ICP between NTG patients and healthy subjects, and seem to disagree with Berdahl et al. (4,5) and Ren et al (6), who reported a significant difference in ICP of 3.1 and 3.4 mmHg, respectively between NTG patients and healthy subjects. There is little research on invasive measurement of ICP in glaucoma (4–6,8,25), so it is difficult to determine the cause of the disagreement. One possible reason for the discrepancy proposed by Linden et al (8) is that there is no difference between the studies in regards to ICP in NTG, but there is, however, a difference

in the control groups. A normal ICP at supine is considered to be between 7-15 mmHg (24) yet, for the 3 studies that showed a difference between groups, the controls had mean ICPs of ~ 13 mmHg which is at the higher end of the normal range. In the study by Ren et al (6) there was a relationship between visual field defects and TLCPD, but in this case the IOP was measured in the upright position while the ICP was measured in the lateral decubitus position. Interestingly, in all studies there was no significant relationship between ICP and severity of visual field defects, suggesting something beyond reduced ICP is responsible for the pathophysiology of NTG. Finally, it could be the case that the role of ICP in glaucoma is limited to a small subgroup of NTG patients, that is, those with an IOP at the lower end of the normal range (~ 10 mmHg; sometimes referred to as low tension glaucoma). These patients were not included in the current study (median sitting IOP 15.4 mmHg; Table 2.1), and also not in the study by Linden et al (7), where the mean sitting IOP was 15.1 mmHg. However, the mean IOP in the studies Berdahl et al (3) and Ren et al (5) were not clearly lower (14.3 and 16.1 mmHg, respectively). Of note, Ren et al included Chinese patients, whereas in the current study and in the study by Linden et al the patients were of Caucasian origin. When looking at the supine position for healthy subjects in the current study, the ICP determined by DPOAE phase increases by 5.5 mmHg and yet IOP increases by only 1.5 mmHg. This suggests that a large change in TLCPD in the supine position, e.g., during sleep, may be part of a normal physiological pattern.

In conclusion, we did not find evidence that NTG patients have a reduced ICP. Beyond glaucoma, however, DPOAEs can be used to monitor ICP changes noninvasively, which may further clinical care and the understanding of ICP variation and regulation tremendously. First, however, future research should focus on a robust and reproducible probe placement in order to minimize variability.

ACKNOWLEDGEMENTS

We would like to thank Paul Avan for very stimulating discussions regarding this study and Echodia for the use of their Elios device and for technical support. We would also like to thank L. van de Waardt, E. Chen Yao, and W. Nieboer for their assistance with data collection.

REFERENCES

1. Casson RJ, Chidlow G, Wood JPM, Crowston JG, Goldberg I. Definition of glaucoma: clinical and experimental concepts. *Clin Experiment Ophthalmol*. 2012;40(4):341–9.
2. Morgan WH, Yu DY, Cooper RL, Alder VA, Cringle SJ, Constable IJ. The influence of cerebrospinal fluid pressure on the lamina cribrosa tissue pressure gradient. *Invest Ophthalmol Vis Sci*. 1995 May;36(6):1163–72.
3. Jonas JB, Ritch R, Panda-Jonas S. Cerebrospinal fluid pressure in the pathogenesis of glaucoma. *Prog Brain Res*. 2015 Sep 9;221:33–47.
4. Berdahl JP, Fautsch MP, Stinnett SS, Allingham RR. Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. *Invest Ophthalmol Vis Sci*. 2008 Dec;49(12):5412–8.
5. Berdahl JP, Allingham RR, Johnson DH. Cerebrospinal fluid pressure is decreased in primary open-angle glaucoma. *Ophthalmology*. 2008 May;115(5):763–8.
6. Ren R, Jonas JB, Tian G, Zhen Y, Ma K, Li S, et al. Cerebrospinal fluid pressure in glaucoma: a prospective study. *Ophthalmology*. 2010 Feb;117(2):259–66.
7. Gallina P, Savastano A, Becattini E, Orlandini S, Scollato A, Rizzo S, et al. Glaucoma in patients with shunt-treated normal pressure hydrocephalus. *J Neurosurg*. 2017 Nov 17;1–7.
8. Lindén C, Qvarlander S, Jóhannesson G, Johansson E, Östlund F, Malm J, et al. Normal-Tension Glaucoma Has Normal Intracranial Pressure: A Prospective Study of Intracranial Pressure and Intraocular Pressure in Different Body Positions. *Ophthalmology* [Internet]. 2017 Oct 30; Available from: <http://dx.doi.org/10.1016/j.ophtha.2017.09.022>
9. Kemp DT. Otoacoustic emissions, their origin in cochlear function, and use. *Br Med Bull*. 2002;63:223–41.
10. Probst R, Lonsbury-Martin BL, Martin GK. A review of otoacoustic emissions. *J Acoust Soc Am*. 1991;89(5):2027–67.
11. Bershad EM, Urfy MZ, Pechacek A, McGrath M, Calvillo E, Horton NJ, et al. Intracranial pressure modulates distortion product otoacoustic emissions: a proof-of-principle study. *Neurosurgery*. 2014 Oct;75(4):445–54; discussion 454–5.
12. Büki B, Chomiccki A, Dordain M, Lemaire JJ, Wit HP, Chazal J, et al. Middle-ear influence on otoacoustic emissions. II: contributions of posture and intracranial pressure. *Hear Res*. 2000 Feb;140(1-2):202–11.
13. Voss SE, Adegoke MF, Horton NJ, Sheth KN, Rosand J, Shera CA. Posture systematically alters ear-canal reflectance and DPOAE properties. *Hear Res*. 2010 May;263(1-2):43–51.
14. Williams MA, Malm J, Eklund A, Horton NJ, Voss SE. Distortion Product Otoacoustic Emissions and Intracranial Pressure During CSF Infusion Testing. *Aerosp Med Hum Perform*. 2016;87(10):844–51.
15. Heeg GP, Blanksma LJ, Hardus PLLJ, Jansonius NM. The Groningen Longitudinal Glaucoma Study. I. Baseline sensitivity and specificity of the frequency doubling perimeter and the GDx nerve fibre analyser. *Acta Ophthalmol Scand*. 2005 Feb;83(1):46–52.

-
-
16. Chaaban MR, Illing E, Riley KO, Woodworth BA. Acetazolamide for high intracranial pressure cerebrospinal fluid leaks. *Int Forum Allergy Rhinol*. 2013 Sep;3(9):718–21.
 17. Gücer G, Viernstein L. Long-term intracranial pressure recording in the management of pseudotumor cerebri. *J Neurosurg*. 1978 Aug;49(2):256–63.
 18. Smith SV, Friedman DI. The Idiopathic Intracranial Hypertension Treatment Trial: A Review of the Outcomes. *Headache*. 2017 Sep;57(8):1303–10.
 19. de Kleine E, Wit HP, van Dijk P, Avan P. The behavior of spontaneous otoacoustic emissions during and after postural changes. *J Acoust Soc Am*. 2000 Jun;107(6):3308–16.
 20. Avan P, Giraudet F, Chauveau B, Gilain L, Mom T. Unstable distortion-product otoacoustic emission phase in Menière's disease. *Hear Res*. 2011;277(1-2):88–95.
 21. Gerenton G, Giraudet F, Djennaoui I, Pavier Y, Gilain L, Mom T, et al. Abnormal fast fluctuations of electrocochleography and otoacoustic emissions in Menière's disease. *Hear Res*. 2015 Sep;327:199–208.
 22. Chapman PH, Cosman ER, Arnold MA. The relationship between ventricular fluid pressure and body position in normal subjects and subjects with shunts. *Neurosurgery*. 1990;181.
 23. Flammer J, Konieczka K, Flammer AJ. The primary vascular dysregulation syndrome: implications for eye diseases. *EPMA J*. 2013 Jun 7;4(1):14.
 24. Albeck MJ, Børgesen SE, Gjerris F, Schmidt JF, Sørensen PS. Intracranial pressure and cerebrospinal fluid outflow conductance in healthy subjects. *J Neurosurg*. 1991 Apr;74(4):597–600.
 25. Pircher A, Remonda L, Weinreb RN, Killer HE. Translaminar pressure in Caucasian normal tension glaucoma patients. *Acta Ophthalmol*. 2017 Nov;95(7):e524–31.
-
-

3

Is acetazolamide an effective treatment for glaucoma? Effects on intraocular and intracranial pressure and trans-lamina cribrosa pressure difference

Allison R. Loiselle, Nomdo M. Jansonius

ABSTRACT

here is a theory that a change in trans-lamina cribrosa pressure difference (TLCPD) could be related to glaucoma. If the difference between intraocular pressure (IOP) and intracranial pressure (ICP) is in fact a link to glaucoma, then medications lowering both pressures simultaneously would be ineffective. Acetazolamide, a carbonic anhydrase inhibitor given to glaucoma patients, is one such medication. We summarized the available research on the effects of acetazolamide on IOP and ICP. There is scarce information about the magnitude and time course of ICP changes elicited by acetazolamide, especially at the doses given to glaucoma patients. Eleven studies for IOP and 3 for ICP were included. The only dose at which there was a crossover in available research for changes in IOP and ICP was 500 mg. At this dose, within 2-6 hours there was an IOP and ICP reduction of 6.2 (95% confidence interval 3.4-9.1) mmHg (24.7%) and 7.4 mmHg (31.5%), respectively. Therefore at this dosage and time frame, the TLCPD would remain essentially unchanged. We argue that the limited evidence summarized here suggests that acetazolamide may not be effective for glaucoma — assuming it is the TLCPD that matters, and not IOP alone — and that future researchers should consider this in their work. A comprehensive understanding of the effect of acetazolamide on ICP and of the TLCPD theory of glaucoma is crucial for glaucoma care.

3.1 BACKGROUND

For decades, treatment for glaucoma has been focused on lowering intraocular pressure (IOP). However, almost half a century ago an intriguing theory was introduced that intracranial pressure (ICP) may play a role in the pathophysiology of glaucoma as well (1). This theory posits that it is not IOP alone, but the interaction between IOP and ICP across the lamina cribrosa (trans-lamina cribrosa pressure difference [TLCPD]) that leads to glaucoma (2). There is some evidence for this theory in which ICP was tested in glaucoma patients, and they were found to have a lower ICP than healthy subjects (3–5), although this was not found in all studies (6–8).

Acetazolamide (AAZ) is a carbonic anhydrase inhibitor (CAI), which is a class of drugs that impairs the resorption of bicarbonate, therefore leading to its excretion along with sodium and water. AAZ is eliminated through the kidneys and the plasma half life is 4-8 hours (9). It is prescribed to glaucoma patients because it decreases the production of aqueous humour, lowering IOP (10–12). At the same time, however, it inhibits enzymes in the choroid plexus and decreases cerebrospinal fluid production, lowering ICP (13). For that reason, it is used in neurology departments.

If the theory that glaucoma patients have a lower ICP than normal is true, and an imbalance in the TLCPD leads to glaucoma, then administration of AAZ may be counterproductive. Due to the considerable side effects of AAZ, it is considered a “second-line” drug, and is prescribed to patients who need acute reduction of extremely high IOPs or who do not respond to first-line medications (14). This, unfortunately, makes it difficult to tease apart the effect of the treatment. If a patient continues to rapidly progress, we may assume that it is due to the severity of their glaucoma, and not that the treatment is insufficient. A randomized controlled trial designed similar to the EMGT (15) or UK glaucoma treatment study (16) could answer the question of whether AAZ is actually effective or harmful in slowing glaucoma progression, but is unlikely to be conducted.

The aim of the current report is (1) to present the available research involving AAZ, IOP, and ICP, and to discuss the potentiality that it is ineffective or, at worst, harmful as a treatment for glaucoma and (2) to uncover gaps in the research that need to be filled in order to solve this problem.

3.2 DISCUSSION

3.2.1 *Acetazolamide and IOP*

In ophthalmology clinics, AAZ is typically prescribed in doses of 125-250 mg two (12 hours apart) or three (8 hours apart) times per day (17). It is administered orally with a tablet. The peak effect typically occurs within 2-4 hours, with an IOP reduction of ~30%, and IOP returns to baseline values within 8 to 12 hours (17, 18). Table 3.1 displays studies demonstrating a reduction in IOP using different doses of AAZ (18–28). The studies concerning IOP changes due to AAZ were compiled using search criteria in PubMed

and Google Scholar of (“acetazolamide” OR “Diamox”) AND (“IOP” OR “Intraocular Pressure”). A control group was not required for inclusion and subjects were a mix of healthy, ocular hypertensives, intracranial hypertensives, and glaucoma patients. Studies using topical CAIs were not included. Since the percentage decrease in IOP or ICP is determined by the initial IOP or ICP, this was also recorded for those studies that reported it. Figure 3.1 demonstrates how the reduction in IOP is highly dependent on the initial pressure.

Even though there is a linear dose-plasma level relationship, the plasma level-IOP relationship may not be linear and the levels associated with peak IOP reduction have a large intersubject variability, as demonstrated in Figure 3.1 (19, 21, 29–31).

3.2.2 Acetazolamide and ICP

The typical dosage given to patients with raised ICP starts at 500 mg/day and can be gradually increased to 1-2 g/day if tolerated (32). However, these doses are not based on invasive measures of actual ICP changes, but on alleviation of visual disturbances that can occur in high ICP patients (32, 33). The improvement of visual problems is assumed to be due to a lowering of the ICP, but information on the actual changes in ICP due to AAZ and the corresponding kinetics is scarce (34). One of the main studies contributing to this area is the idiopathic intracranial hypertension (IIH) treatment trial (IIHTT) (35). In this multi-site study, 165 patients with IIH were randomly assigned to either receive AAZ or a placebo, and both groups also received a supervised diet. The patients were given a starting dose of 250 mg/day which was then slowly increased until it was no longer tolerated, for many patients this was up to 4 g/day. After 6 months, 85 patients elected to have a second lumbar puncture which revealed a reduction of 31.3% (from 26.2 to 18.0 mmHg) in ICP for the AAZ group (n=47) compared with a 14.5% reduction (from 26.2 to 22.4 mmHg) in the placebo group (36). IOP was also measured in this study, yielding a small, non-significant decrease in the AAZ group (from 14.8 to 14.4 mmHg) and no change in the placebo group (M. Wall, personal correspondence, June 27, 2018). In another study by Chaaban et al (37), 36 patients received 500 mg of AAZ and 4-6 hours later there was a 31.5% reduction (from 23.5 to 16.1 mmHg) in ICP.

Due to the difficulty of continuous ICP monitoring, only one study shows how AAZ affects ICP over time. Gucer et al (38) used a small pressure sensing device embedded in the skull to continuously measure ICP in 4 patients. In one of these patients, a first dose of 4 g led to a reduction in ICP of nearly 50% and after 8-12 hours it was still reduced. As previously mentioned, IOP returns to baseline values within 8-12 hours, but there are no corresponding time course studies for ICP using the much smaller doses given for IOP reduction. There is no reason to suspect that the relationship between plasma levels of AAZ and ICP reduction would differ from that of IOP, but with so little evidence it is difficult to be certain.

Table 3.1 | Reduction in IOP with acetazolamide

Study	N	Subject Type	Time since dose (hrs)	Initial IOP (mmHg; Mean $\pm$ SD*)	IOP reduction (mmHg; Mean $\pm$ SD*)	IOP reduction (%; Mean $\pm$ SD*)
Dose: 62.5 mg						
Friedland et al (1977)	9	OHT	2	27.5	8.2 $\pm$ 2.2	29.8
Dose: 125 mg						
Friedland et al (1977)	9	OHT	2	27.5	8.0 $\pm$ 2.2	29.1
Ellis et al (1982)	9	OHT	2	>22	NA	23.0 $\pm$ 6.1
Dose: 187.5 mg						
Alm et al (1982)†	10	POAG/PEX	Average (1-11)‡	31.1 $\pm$ 2.1	3.0 $\pm$ 2.5	9.6
Dose: 250 mg						
Friedland et al (1977)	9	OHT	2	27.5	8.4 $\pm$ 1.6	30.5
Ellis et al (1982)	9	OHT	2	>22	NA	26.9 $\pm$ 5.2
Rosenberg et al (1998)†	4	OHT/POAG	3	20.5 $\pm$ 2.2	3.6	17.5
Rulo et al (1997)†	24	POAG	4	25.1 $\pm$ 5.2	4.6	18.3
Maus et al (1997)†	39	Healthy	4	12.5 $\pm$ 2.2	2.4	19.2
Dose: 375 mg						
Alm et al (1982)†	10	POAG/PEX	Average (1-11)‡	31.1 $\pm$ 2.1	5.1 $\pm$ 2.5	16.4
Dose: 500 mg						
Macdonald et al (1977)	8	POAG/PCAG	5	20.0	3.5	17.5
Friedland et al (1977)	9	OHT	2	27.5	9.3 $\pm$ 1.7	33.8
Becker et al (1954)	3	POAG	3-5	35.3 $\pm$ 8.5	15.5 $\pm$ 6.1	43.6 $\pm$ 11.4
Becker et al (1954)	4	Healthy	3-5	17.0 $\pm$ 1.4	3.5 $\pm$ 1.2	21.1 $\pm$ 7.5
Kass et al (1982)	10	OHT	12§	28.1 $\pm$ 6.3	9.0	32.0
Bietti et al (1975)	8	POAG/Healthy	4	32.0	9.0	28.1

Table 3.1 | Continued.

Study	N	Subject Type	Time since dose (hrs)	Initial IOP (mmHg; Mean $\pm$ SD*)	IOP reduction (mmHg; Mean $\pm$ SD*)	IOP reduction (%; Mean $\pm$ SD*)
Zinkernagel & Ebner (2009)	17	POAG	2	18.4 $\pm$ 2.7	2.7 $\pm$ 2.9	14.6 $\pm$ 11.5
Zinkernagel & Ebner (2009)	22	Healthy	2	16.3 $\pm$ 2.5	3.2 $\pm$ 1.9	19.8 $\pm$ 7.6
Alm et al (1982)†	9	POAG/PEX	Dose: 750 mg Average (1-11)‡	31.1 $\pm$ 2.1	7.7 $\pm$ 3.3	24.7
Alm et al (1982)†	8	POAG/PEX	Dose: 1000 mg Average (1-11)‡	31.1 $\pm$ 2.1	6.8 $\pm$ 3.4	21.9
Becker et al (1954)	1	POAG	3-5	60.0	36.0	60.0

* Standard deviation shown when provided in the article or when it could be calculated from available information

† Measurements were completed after continuous use for 1 day up to 1 week, all other studies were single dose

‡ Average of 7 IOP measurements across the day, individual hours not available

§ Sustained release tablets

OHT: Ocular Hypertension; PEX: Pseudoexfoliative Glaucoma; POAG: Primary Open Angle Glaucoma; PCAG: Primary closed angle glaucoma.

FIG 3.1 Reduction in IOP after different doses of AAZ compared with initial pressure. This demonstrates the strong relationship between the two.

3.2.3 Acetazolamide and TLCPD

The only dose at which there was a crossover in available data for changes in IOP and ICP was 500 mg. At this dose, within 2-6 hours there was an IOP reduction of 6.2 mmHg (sample size weighted mean reduction from Table 3.1 500 mg studies; 95% confidence interval [CI] 3.4-9.1 mmHg) and an ICP reduction of 7.4 mmHg (37). The corresponding relative reductions were 24.7% (95% CI 18.3-31.1%) and 31.5%, respectively.

If IOP alone affects glaucoma and ICP plays no role, then AAZ is an effective drug. It significantly reduces IOP, and can be used in patients who have not been responsive to other medications or treatment modalities (14). If ICP does indeed play a role in the pathophysiology of glaucoma, which would make the TLCPD the target for treatment, then the ICP lowering effects of AAZ may be counterproductive.

As mentioned above, the only dose at which there was a crossover in available research for changes in IOP and ICP was at 500 mg. At this dose and time frame, the TLCPD would remain essentially unchanged. This problem could be further exacerbated by diurnal fluctuations (39), especially if the diurnal changes in IOP differ from those of ICP. It is possible that the relative effects of AAZ may differ between normal IOP, high ICP conditions (like IIH) and high IOP, normal ICP conditions (like glaucoma). We therefore cannot assume that glaucoma patients would have as dramatic a decrease in ICP as a patient with IIH. Figure 3.1 shows that the change in IOP strongly depends on baseline IOP (much stronger

than it depends on dose); corresponding data for ICP are not available. The limited evidence summarized here seems at best to suggest that AAZ may not be effective and, at worst, could be harmful — assuming it is the TLCPD that matters, and not IOP alone.

3.3 CONCLUSION

Given the many uncertainties, the only way to truly prove or disprove a beneficial effect on glaucoma progression would be a randomized control trial. Such a trial would take many years and require a large number of subjects and is therefore unlikely to happen. Therefore a comprehensive understanding of the effect of AAZ on ICP and of the TLCPD theory of glaucoma are crucial, but currently lacking. Determination of an accurate noninvasive method for ICP assessment (8, 40, 41) is essential to facilitate studies with lower costs and larger sample sizes, thus furthering this field and filling the gaps in the current research.

3

REFERENCES

1. Volkov VV. [Essential element of the glaucomatous process neglected in clinical practice]. *Oftalmol Zh.* 1976;31(7):500–4.
2. Jonas JB, Wang N, Yang D, Ritch R, Panda-Jonas S. Facts and myths of cerebrospinal fluid pressure for the physiology of the eye. *Prog Retin Eye Res.* 2015 May;46:67–83.
3. Berdahl JP, Fautsch MP, Stinnett SS, Allingham RR. Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. *Invest Ophthalmol Vis Sci.* 2008 Dec;49(12):5412–8.
4. Berdahl JP, Allingham RR, Johnson DH. Cerebrospinal fluid pressure is decreased in primary open-angle glaucoma. *Ophthalmology.* 2008 May;115(5):763–8.
5. Ren R, Jonas JB, Tian G, Zhen Y, Ma K, Li S, et al. Cerebrospinal fluid pressure in glaucoma: a prospective study. *Ophthalmology.* 2010 Feb;117(2):259–66.
6. Lindén C, Qvarlander S, Jóhannesson G, Johansson E, Östlund F, Malm J, et al. Normal-Tension Glaucoma Has Normal Intracranial Pressure: A Prospective Study of Intracranial Pressure and Intraocular Pressure in Different Body Positions. *Ophthalmology.* 2018 Mar;125(3):361–8.
7. Pircher A, Remonda L, Weinreb RN, Killer HE. Translaminar pressure in Caucasian normal tension glaucoma patients. *Acta Ophthalmol.* 2017 Nov;95(7):e524–31.
8. Loissele AR, de Kleine E, van Dijk P, Jansonius NM. Noninvasive intracranial pressure assessment using otoacoustic emissions: An application in glaucoma. *PLoS One.* 2018 Oct 1;13(10):e0204939.
9. Van Berkel MA, Elefritz JL. Evaluating off-label uses of acetazolamide. *Am J Health Syst Pharm.* 2018 Apr 15;75(8):524–31.
10. Costagliola C, dell’Omo R, Romano MR, Rinaldi M, Zeppa L, Parmeggiani F. Pharmacotherapy of intraocular pressure - part II. Carbonic anhydrase inhibitors, prostaglandin analogues and prostamides. *Expert Opin Pharmacother.* 2009 Dec;10(17):2859–70.
11. Stamper RL, Lieberman MF, Drake MV. Carbonic anhydrase inhibitors. In: Becker-Shaffer’s *Diagnosis and Therapy of the Glaucomas.* 2009. p. 407–19.
12. Maren TH. Carbonic anhydrase: General perspective and advances in glaucoma research. *Drug Dev Res.* 1987;10(4):255–76.
13. Supuran CT. Acetazolamide for the treatment of idiopathic intracranial hypertension. *Expert Rev Neurother.* 2015 Jul 7;15(8):851–6.
14. Lee DA, Higginbotham EJ. Glaucoma and its treatment: a review. *Am J Health Syst Pharm.* 2005 Apr 1;62(7):691–9.
15. Leske MC, Heijl A, Hyman L, Bengtsson B. Early manifest glaucoma trial. *Ophthalmology.* 1999;106(11):2144–53.
16. Garway-Heath DF, Lascaratos G, Bunce C, Crabb DP, Russell RA, Shah A. The United Kingdom Glaucoma Treatment Study. *Ophthalmology.* 2013;120(1):68–76.
17. ACETAZOLAMIDE in glaucoma. *Br Med J.* 1955 Aug 20;2(4937):479–81.
18. Becker B. Diamox and the therapy of glaucoma. *Am J Ophthalmol.* 1954 Jul;38(11):109–11.
19. Friedland BR, Mallonee J, Anderson DR. Short-term dose response characteristics of acetazolamide in man. *Arch Ophthalmol.* 1977 Oct;95(10):1809–12.
20. Ellis PP, Price PK, Kelmenson R, Rendi MA. Effectiveness of generic acetazolamide. *Arch Ophthalmol.* 1982 Dec;100(12):1920–2.
21. Alm A, Berggren L, Hartvig P, Roosdorp M. Monitoring acetazolamide treatment. *Acta Ophthalmol.* 1982 Feb;60(1):24–34.
22. Rosenberg LF, Krupin T, Tang L-Q, Hong PH, Ruderman JM. Combination of systemic acetazolamide and topical dorzolamide in reducing intraocular pressure and aqueous humor formation. *Ophthalmology.* 1998;105(1):88–93.

23. Rulo AH, Greve EL, Hoyng PFJ. Additive Ocular Hypotensive Effect of Latanoprost and Acetazolamide. *Ophthalmology*. 1997;104(9):1503–7.
24. Maus TL, Larsson LI, McLaren JW, Brubaker RF. Comparison of dorzolamide and acetazolamide as suppressors of aqueous humor flow in humans. *Arch Ophthalmol*. 1997 Jan;115(1):45–9.
25. Macdonald MJ, Gore SM, Cullen PM, Phillips CI. Comparison of ocular hypotensive effects of acetazolamide and atenolol. *Br J Ophthalmol*. 1977 May;61(5):345–8.
26. Kass MA, Korey M, Gordon M, Becker B. Timolol and acetazolamide. A study of concurrent administration. *Arch Ophthalmol*. 1982 Jun;100(6):941–2.
27. Bietti G, Virno M, Pecori-Giraldi J. Acetazolamide, metabolic acidosis, and intraocular pressure. *Am J Ophthalmol*. 1975 Sep;80(3 Pt 1):360–9.
28. Zinkernagel MS, Ebnetter A. Acetazolamide influences ocular pulse amplitude. *J Ocul Pharmacol Ther*. 2009 Apr;25(2):141–4.
29. Yano I, Takayama A, Takano M, Inatani M, Tanihara H, Ogura Y, et al. Pharmacokinetics and pharmacodynamics of acetazolamide in patients with transient intraocular pressure elevation. *Eur J Clin Pharmacol*. 1998 Mar;54(1):63–8.
30. Berson FG, Epstein DL, Grant WM, Hutchinson BT, Dobbs PC. Acetazolamide dosage forms in the treatment of glaucoma. *Arch Ophthalmol*. 1980 Jun;98(6):1051–4.
31. Joyce PW, Mills KB. Comparison of the effect of acetazolamide tablets and sustets on diurnal intraocular pressure in patients with chronic simple glaucoma. *Br J Ophthalmol*. 1990 Jul;74(7):413–6.
32. Friedman DI, Jacobson DM. Idiopathic intracranial hypertension. *J Neuroophthalmol*. 2004 Jun;24(2):138–45.
33. Smith SV, Friedman DI. The Idiopathic Intracranial Hypertension Treatment Trial: A Review of the Outcomes. *Headache*. 2017 Sep;57(8):1303–10.
34. Piper RJ, Kalyvas AV, Young AMH, Hughes MA, Jamjoom AAB, Fouyas IP. Interventions for idiopathic intracranial hypertension. *Cochrane Database Syst Rev*. 2015 Aug 7;(8):CD003434.
35. Friedman DI, McDermott MP, Kiebertz K, Kupersmith M, Stoutenburg A, Keltner JL, et al. The idiopathic intracranial hypertension treatment trial: design considerations and methods. *J Neuroophthalmol*. 2014 Jun;34(2):107–17.
36. Kattah JC, Pula JH, Mejico LJ, McDermott MP, Kupersmith MJ, Wall M. CSF pressure, papilledema grade, and response to acetazolamide in the Idiopathic Intracranial Hypertension Treatment Trial. *J Neurol*. 2015 Oct;262(10):2271–4.
37. Chaaban MR, Illing E, Riley KO, Woodworth BA. Acetazolamide for high intracranial pressure cerebrospinal fluid leaks. *Int Forum Allergy Rhinol*. 2013;3(9):718–21.
38. Gücer G, Viernstein L. Long-term intracranial pressure recording in the management of pseudotumor cerebri. *J Neurosurg*. 1978 Aug;49(2):256–63.
39. Wostyn P, De Groot V, Van Dam D, Audenaert K, De Deyn PP. Intracranial pressure fluctuations: a potential risk factor for glaucoma? *Acta Ophthalmol*. 2013;93(1):e83–4.
40. Williams MA, Malm J, Eklund A, Horton NJ, Voss SE. Distortion Product Otoacoustic Emissions and Intracranial Pressure During CSF Infusion Testing. *Aerosp Med Hum Perform*. 2016;87(10):844–51.
41. Robba C, Bacigaluppi S, Cardim D, Donnelly J, Bertuccio A, Czosnyka M. Non-invasive assessment of intracranial pressure. *Acta Neurol Scand*. 2016 Jul;134(1):4–21.

4

Intraocular and intracranial pressure in glaucoma patients taking acetazolamide

Allison R. Loisel, Emile de Kleine, Pim van Dijk, Nomdo M. Jansonius

(Published: PLoS ONE. June 2020; 15(6):e0234690)

ABSTRACT

The alternative mechanical theory of glaucoma, in which an increased pressure difference across the lamina cribrosa (difference between intraocular and intracranial pressure; IOP and ICP), rather than solely an elevated IOP, leads to structural and functional vision loss, is still controversial. If the theory is true, a drug that simultaneously lowers both the IOP and ICP may be ineffective. The aim of this study was to determine how acetazolamide (AAZ; a drug prescribed in glaucoma that aims to lower the IOP) affects both IOP and ICP in glaucoma patients and to compare the magnitude and time course of the induced pressure changes with those of healthy subjects not taking AAZ. IOP and noninvasive ICP (measured through emissions from the ear) were measured in 20 glaucoma patients taking 125 mg of AAZ twice daily. Measurements were taken for 30 minutes before taking the drug and for 2 hours post-ingestion. Comparisons were made with 13 age-similar controls. After 12 hours with no anti-glaucoma medication, AAZ did not further reduce IOP in glaucoma patients compared to controls ($P=0.58$) but did reduce ICP compared to controls ($P=0.035$), by approximately 4 mmHg. Our findings suggest that there are periods during the day when the pressure difference across the lamina cribrosa is larger in case of AAZ use. Future studies should focus on improving the noninvasive ICP testing, different doses and dosing schedules of AAZ, and the time course of IOP in glaucoma patients not taking AAZ.

4.1 INTRODUCTION

Glaucoma is a chronic and progressive eye disease characterized by loss of retinal ganglion cells, thinning of the retinal nerve fiber layer, and subsequent visual field loss. If left untreated, it can eventually lead to blindness. Currently, a high intraocular pressure (IOP) is the only treatable factor in the pathophysiology of glaucoma. However, many patients continue to progress after IOP is controlled and others have normal tension glaucoma (NTG) in which their IOP is normal even before treatment (1), suggesting another mechanism is needed to explain the disease. One possible theory is that glaucoma patients have a reduction in intracranial pressure (ICP) (2–4). But this has not been confirmed in some more recent studies (5–7).

The lamina cribrosa is a porous layer at the back of the eye through which nerve fibers run. In the retrobulbar space, cerebrospinal fluid surrounds the optic nerve and ICP is therefore transferred to this area and can act on the lamina cribrosa. The idea behind the ICP theory of glaucoma is that both the IOP and ICP interact at the posterior part of the eye and, if not in balance, can cause mechanical stress and therefore nerve fiber damage (8). This balance can be investigated by measuring the trans-lamina cribrosa pressure difference (TLCPD), or the difference between the IOP and the ICP at the level of the lamina cribrosa.

Acetazolamide (AAZ) is a carbonic anhydrase inhibitor that is used in glaucoma treatment to lower IOP, by a mechanism of lowering aqueous humor production (9–12). However, it is also used in the treatment of high ICP, because it inhibits enzymes in the choroid plexus and decreases production of cerebrospinal fluid (13–16). If it is true that the TLCPD is a causal factor in the incidence or progression of glaucoma, then a drug that simultaneously lowers both IOP and ICP may not be effective. In fact, if the magnitude of the ICP change is *more* than that of the IOP, it could actually be harmful.

While research on the effects of AAZ on IOP exists (17–22), unfortunately, there is little information about the effects of AAZ on ICP. In the majority of the neurological clinical cases, patients' doses are increased until complaints — like headache or double vision — are alleviated. There is an assumed reduction in ICP, but the absolute change and time course are not well elucidated. Although some research has been completed (16,23,24), the effect of AAZ on ICP on the scale of hours needs further investigation. This can be difficult because the current gold standard for ICP measurement is the lumbar puncture, which is cumbersome and painful for the patient and is not optimal for continuous measurement. Noninvasive methods of ICP measurement like distortion product otoacoustic emissions (DPOAEs), which can be measured continuously, are therefore of great interest.

DPOAEs are emitted by the inner ear in response to two tones at specified levels and frequencies. The emissions are dependent on ICP because of the connection between the fluid in the intracranial space and the inner ear fluid through the endolymphatic and cochlear ducts. Changes in ICP cause pressure changes on the stapes and therefore on the transmission of sound through the middle ear. DPOAEs have already been shown to accurately represent ICP (25–28). Although DPOAEs are not yet able to give absolute pressure readings, they can show changes in ICP, due to changing body positions or the use

of drugs like AAZ. We recently calibrated the relationship between DPOAE change and ICP change by comparing the body-position dependency of DPOAEs with that of ICP (5,6).

If AAZ lowers the IOP and ICP such that the TLCPD remains unchanged, it may be an ineffective treatment for glaucoma. The aim of the current study is therefore to determine the TLCPD in glaucoma patients taking AAZ by measuring IOP and ICP noninvasively and comparing the magnitude and time course of the induced pressure changes with those of healthy subjects not taking AAZ.

4.2 METHODS

4.2.1 Study population

Subjects with healthy eyes who responded to our advertisement and glaucoma patients who were selected based on the inclusion criteria (listed below) from the Groningen Longitudinal Glaucoma Study database (29), received an information letter and informed consent form. The ethics board of the University Medical Center Groningen (UMCG) approved the study protocol. All participants provided written informed consent. The study followed the tenets of the Declaration of Helsinki.

The methods of this study were similar to a previous study from our lab (6). In short, in order to be eligible to participate in this study, all subjects had to meet the following inclusion criteria: 50 to 70 years of age and detectable DPOAEs in at least one ear. Additionally, for controls: upright IOP of 21 mmHg or lower, no eye disease, and no family history of glaucoma (determined by a questionnaire). To exclude eye disease, we performed optical coherence tomography (OCT-HS100; Canon, Tokyo, Japan; considered normal if the mean retinal nerve fiber layer and retinal ganglion cell layer thickness in macular area were above the 5th percentile), frequency doubling technology (FDT; Carl Zeiss, Jena, Germany; no reproducibly abnormal test locations allowed in C20-1 screening mode), and a measurement of visual acuity (visual acuity at least 0.8 in both eyes). For patients, glaucoma was defined according to Heeg et al (29). We required a reproducible (same hemifield and at least partially overlapping) visual field defect (Humphrey Field Analyzer 30-2 SITA fast; Carl Zeiss, Jena, Germany; criterion: 'glaucoma hemifield test' outside normal limits) in at least one eye that had to be compatible with glaucoma and without any other explanation. In contrast to our previous study, the patients could include those with primary open angle (POAG), secondary, and pigment dispersion glaucoma. All patients took 125 mg of AAZ for the study, and had a minimum of 12 hours since their last regular dose, which was 125 mg twice daily in all cases. Any IOP-lowering eye drops were continued but the morning dose, if applicable, was delayed until the end of the study-related measurements, which were performed around the morning dose of AAZ (see below for details).

4.2.2 DPOAE parameters

DPOAEs were measured using hardware (Elios) and software (Echosoftware version 2.1.8.1) developed by Echodia (St. Beaupré, France). In order to ensure the highest magnitude at the $2f_1-f_2$ emission, we used a fixed rate of $f_2/f_1=1.20$ with tones at frequencies $f_1=1000$ Hz and $f_2=1200$ Hz and levels $L_1=L_2=70$ dB SPL. All DPOAE measurements were completed in a sound-isolated audiometric room in the otorhinolaryngology clinic.

4.2.3 Measurement Protocol

Blood pressure (Omron Model M6 Comfort, Omron Healthcare Co., Ltd.) and upright IOP in both eyes (iCare Pro tonometer, Icare Finland Oy) were measured. Subjects then laid down on an airbed and IOP was measured once again in the supine position.

For DPOAEs, the ear with the highest signal to noise ratio (SNR) was used for testing. DPOAEs were measured after 1 minute in the supine position, and then subsequently every 15 minutes. After 30 minutes in the supine position, patients were asked to take their AAZ, and healthy subjects were asked to simply sit up and take a sip of water. The three measurements taken before drug administration were averaged to create a baseline. After this, measurements resumed in supine position every 15 minutes for the remaining 2 hours. For each test, 10 DPOAE measurements were taken over approximately 30 seconds. IOP was measured immediately after each DPOAE test. Blood pressure was also measured 30 and 90 minutes after AAZ intake for patients and after the healthy subjects sat up to drink water.

4.2.4 Data analysis

The subject characteristics were described with mean and standard deviation (SD) for normally distributed variables. For variables with a skewed distribution, we used median and interquartile range (IQR) instead. Groups were compared using the student's t-test and proportions were compared using a chi-square test.

The stimulus and data collection protocols have already been described in detail (30). From the 10 DPOAE measurements taken at each 15 minute interval, those with a SNR of less than 3 dB were excluded after which the remaining measurements were averaged. For healthy subjects and patients with bilateral glaucoma, the IOP of both eyes was averaged. For those with unilateral glaucoma (4 out of 21 patients), only the affected eye was used. All participants started the protocol in the morning between 8:30 and 10:00 to make the inevitable influences of diurnal fluctuations as similar as possible within and between the groups.

Due to the subject specific, unknown offset of the DPOAE phase (the quantity provided by the device), data were presented relative to the calculated baseline value. Next, the resulting DPOAE phase shift was converted into an ICP change from baseline using a conversion factor of 4 degrees/mmHg (6). From the time series of ICP measurements (each 15 minutes during two hours, with the AAZ intake in patients and the sip of water in controls at $t=0$), we determined a trend by using a linear regression for each subject. A one-sided t-test was used to compare the resulting slopes between patients and controls (H_0 : AAZ has no influence on ICP; H_1 : AAZ lowers ICP). The difference in the slope of the ICP

change between patients and controls was considered to be the AAZ effect on ICP. A similar comparison between the groups was made for IOP. Finally, the ICP slopes were compared to the IOP slopes in the patients, using a two-sided paired t-test (H_0 : effect of AAZ on ICP equals effect on IOP; H_1 : effect of AAZ is different for ICP and IOP).

All analyses were performed using R (version 3.3.3; R Foundation for Statistical Computing, Vienna, Austria). A P-value of 0.05 or less was considered statistically significant.

4.3 RESULTS

The characteristics of the study population can be found in Table 4.1. From the 21 patients and 14 controls tested, one patient and one control had to be removed from the analysis due to low signal to noise ratios during multiple measurements. Therefore, 20 patients and 13 healthy subjects were used for analysis. The groups were age- and gender-similar and, despite the patients stopping all anti-glaucoma drugs for a minimum of 12 hours before the study, there was no difference in upright IOP measurements.

Table 4.1 | Characteristics of the study population

	Glaucoma n = 20	Healthy n = 13	P-value
Age (yrs)	61.4 ± 5.2	59.5 ± 6.6	0.38
Gender (% female)	35.0%	61.5%	0.44
Supine SBP (mmHg)	130.4 ± 13.3	122.7 ± 15.7	0.30
Supine DBP (mmHg)	82.6 ± 7.4	75.4 ± 10.7	0.17
VF MD better eye (dB; median [IQR])	-13.1 (-4.4 to -18.8)	NA	-
VF MD worse eye (dB; median [IQR])	-25.7 (-14.3 to -27.7)	NA	-
Pre-treatment IOP (mmHg; median [IQR])	32.0 (25 to 38)	NA	-
Upright IOP (mmHg)	16.6 ± 5.3	16.1 ± 3.4	0.59

SBP Systolic blood pressure, *DBP* Diastolic blood pressure, *VF MD* standard automated perimetry mean deviation, *IOP* intraocular pressure.

Figure 4.1 shows the effect of AAZ on DPOAE phase in glaucoma patients compared to controls from baseline until 2 hours post-drug, when the expected peak effect should occur. A negative phase shift means a lower ICP. There was a significant difference in ICP (the AAZ effect on ICP) between the groups ($P=0.035$). Given the conversion factor mentioned above, the observed AAZ effect on ICP from baseline to the final 2 measurements of the 2 hour measurement period post AAZ ingestion would equate to approximately a 4 mmHg decrease in ICP for patients compared to controls (6).

FIG 4.1 Effect of AAZ on DPOAE phase (mean $\pm$ standard error) in glaucoma patients ($n=20$) compared to controls not taking AAZ ($n=13$). ICP on the secondary y-axis was calculated from the DPOAE: based on a previously determined relationship of $4^\circ/\text{mmHg}$ (6). Time ($t=0$) was the calculated baseline value and the drug administration time for patients.

Figure 4.2 shows the effect of AAZ on IOP in glaucoma patients compared to the IOP in controls, not taking AAZ. There was no difference in IOP change between the groups ($P=0.58$). Despite the apparent trend that AAZ had a greater effect on ICP than IOP, the AAZ induced change of ICP versus IOP in patients did not differ significantly ($P=0.085$). Figure 4.3 shows ICP and IOP in patients at 2 hours post ingestion, relative to baseline. The comparison utilizes the previously mentioned 4 degrees/mmHg conversion so that both pressures are expressed in the same unit.

FIG 4.2 Effect of AAZ on IOP (mean $\pm$ standard error) in glaucoma patients ($n=20$) compared to controls not taking AAZ ($n=13$). Time ($t=0$) was the calculated baseline value and the drug administration time for patients.

FIG 4.3 Difference in ICP and IOP from baseline to 2 hours post-AAZ ingestion in glaucoma patients ($n=20$).

4.4 DISCUSSION

In the current study, we find that after 12 hours with no anti-glaucoma medication, 125 mg of AAZ does not further reduce IOP in glaucoma patients, but does reduce ICP. Controls did not show a reduction, suggesting that the ICP reduction in glaucoma patients indeed is an AAZ effect and not a diurnal one.

The IOP lowering effect of AAZ was already demonstrated 65 years ago when Becker et al (11) showed that IOP was reduced 43.6% in POAG patients within 3-5 hours of taking 250 mg of AAZ. Subsequent studies have shown reductions in IOP between 15-34% in patients with POAG and ocular hypertension within hours after varying doses of AAZ (19,20,22,31,32). At a dose of 125 mg — the dose used in the current study — IOP was reduced by 29% in OHT patients within 2 hours (19). In the current study, IOP did not significantly change from baseline to 2 hours after taking AAZ. A major difference between the earlier studies and our study is that in the above-cited studies they used a one-time administration whereas we studied IOP before and after a regular intake. Dose intervals are normally chosen to minimize plasma level fluctuations (with an interval shorter than the half life of the drug), but this is not the case for AAZ twice daily (half life of AAZ: 8 hours). If the half life of a drug equals the dose interval, the plasma level during continuous dosing ranges from $c_{\max}$ to $2c_{\max}$, with a mean plasma level of $1.44c_{\max}$ (assuming first order kinetics), where $c_{\max}$ is the peak plasma level (concentration) after a one-time administration. As such, the expected AAZ plasma level fluctuation during continuous dosing of 125 mg every 8 hours would equal the peak plasma level after a one-time administration of 125 mg. Obviously, the effect of a drug is not directly proportional to the plasma level, at some level saturation occurs. A better formulation is therefore that the expected effect of AAZ on IOP during continuous dosing of 125 mg every 8 hours would equal the difference in effect between a one-time administration of 250 mg and a one-time administration of 125 mg. Given that this difference in effect has been observed (18–20), we had expected to observe an effect of AAZ on IOP in our study design, especially because the plasma level fluctuation of 125 mg every 12 hours should be even larger than that of 125 mg every 8 hours.

Another possible explanation for the limited effect on IOP is that there may have been some influence from the topical IOP lowering drugs that some patients were taking. Patients continued using these drugs uninterrupted (see below), except for delaying any morning dose, if applicable, until the end of the current study. The effect of two IOP-lowering drugs together is smaller than the sum of the individual effects (33) and, for topical medication, this might influence IOP more than ICP. As all but one of our patients used topical IOP-lowering drugs alongside AAZ, we could not test this hypothesis. Finally, glaucoma has been reported to increase diurnal fluctuations in IOP. The IOP pattern during the day varies between and even within individual patients, but, on average, IOP tends to peak in the morning (34,35). As such, our flat IOP curve in the glaucoma patients might actually reflect a successful treatment of the morning peak. A control group of glaucoma patients not taking AAZ would have been helpful here.

While there is ample literature describing the effects of AAZ on IOP, this is not the case for its effect on ICP. Since lumbar punctures are invasive and painful, there is a lack

of data on healthy subjects. Lumbar punctures also do not work well for continuous ICP measurement, so very little is known about hourly changes, both from normal diurnal fluctuations and those induced by medications. The only dose of AAZ at which there was a crossover in available data for changes in IOP and ICP was 500 mg. In a study of 36 patients with cerebrospinal fluid leaks who were given 500 mg of AAZ, there was an ICP reduction of 7.4 mmHg within 4-6 hours of administration (24). This is a reduction of 31.5%, which is similar to that seen in IOP. In our study, the greatest reduction in DPOAE phase was 14.8 degrees in patients taking AAZ. By using the previously defined ratio of 4 degrees of phase shift per mmHg of ICP change, this would equate to an ICP reduction of 3.7 mmHg. This value makes physiological sense in comparison to the study from Chaaban et al. because the patients in the current study did not start with a high ICP, were given a lower AAZ dose, and got their dose as part of an ongoing treatment with AAZ twice daily.

A limitation in this study was the above mentioned inability to implement a “wash-out” period for patients to make sure that no previously taken IOP lowering medications could affect the results. Stopping their drugs for a longer period was not possible due to the ethical limitations on asking patients to stop treatment. However, measuring IOP and ICP during chronic use of IOP-lowering drugs is more representative of the real life pressure changes experienced by patients. All subjects in this study were taking 125 mg of AAZ twice daily, as this is the most common dose given at our clinic for a long period of time. It is possible that higher doses may have elicited a change in IOP with or without a more marked reduction in ICP. A strong point in this study is that we were able to use a noninvasive measurement technique to measure IOP and ICP in glaucoma patients for the first time, at an unprecedented resolution in time. However, while the test was simple and comfortable for patients, the intersubject variability in DPOAE phase responses may limit the ability to accurately assess ICP changes in individual patients.

What are the clinical implications of our observations? If ICP does indeed play a role in the pathophysiology of glaucoma, which would make the TLCPD the target for treatment, then the ICP lowering effects of AAZ may be counterproductive, when IOP remains constant. In this study, we showed that ICP may fluctuate by about 4 mmHg even at an AAZ dose of only 125 mg twice daily. This would suggest that while IOP reaches a plateau during treatment with AAZ, ICP may dip hours after ingestion and create periods during the day when the TLCPD is larger. However, it could also be the case that the TLCPD with chronic AAZ therapy is lower than without, except for a few hours after the AAZ dose. The mean effect of AAZ on IOP would then exceed the mean effect on ICP but with less fluctuation for IOP.

In conclusion, this study showed that glaucoma patients taking AAZ long-term did not have a further IOP reduction immediately after taking their normal AAZ dose of 125 mg, however, their ICP did decrease during this time compared to healthy subjects. Future studies should focus on improving the efficacy of noninvasive ICP testing and on absolute measurements. Results both with and without a wash-out period are needed, results with other AAZ doses and dosing schedules, and results in glaucoma patients not taking AAZ, to better determine if AAZ is helpful or harmful in glaucoma.

ACKNOWLEDGMENTS

We would like to thank F. Aanstoot for her assistance with data collection.

REFERENCES

1. Casson RJ, Chidlow G, Wood JPM, Crowston JG, Goldberg I. Definition of glaucoma: clinical and experimental concepts. *Clin Experiment Ophthalmol*. 2012 May;40(4):341–9.
2. Berdahl JP, Allingham RR, Johnson DH. Cerebrospinal fluid pressure is decreased in primary open-angle glaucoma. *Ophthalmology*. 2008 May;115(5):763–8.
3. Berdahl JP, Fautsch MP, Stinnett SS, Allingham RR. Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. *Invest Ophthalmol Vis Sci*. 2008 Dec;49(12):5412–8.
4. Ren R, Jonas JB, Tian G, Zhen Y, Ma K, Li S, et al. Cerebrospinal fluid pressure in glaucoma: a prospective study. *Ophthalmology*. 2010 Feb;117(2):259–66.
5. Lindén C, Qvarlander S, Jóhannesson G, Johansson E, Östlund F, Malm J, et al. Normal-Tension Glaucoma Has Normal Intracranial Pressure: A Prospective Study of Intracranial Pressure and Intraocular Pressure in Different Body Positions. *Ophthalmology*. 2018 Mar;125(3):361–8.
6. Loissele AR, de Kleine E, van Dijk P, Jansonius NM. Noninvasive intracranial pressure assessment using otoacoustic emissions: An application in glaucoma. *PLoS One*. 2018 Oct 1;13(10):e0204939.
7. Pircher A, Remonda L, Weinreb RN, Killer HE. Translaminar pressure in Caucasian normal tension glaucoma patients. *Acta Ophthalmol*. 2017 Nov;95(7):e524–31.
8. Jonas JB, Ritch R, Panda-Jonas S. Cerebrospinal fluid pressure in the pathogenesis of glaucoma. *Prog Brain Res*. 2015 Sep 9;221:33–47.
9. Costagliola C, dell’Omo R, Romano MR, Rinaldi M, Zeppa L, Parmeggiani F. Pharmacotherapy of intraocular pressure - part II. Carbonic anhydrase inhibitors, prostaglandin analogues and prostamides. *Expert Opin Pharmacother*. 2009 Dec;10(17):2859–70.
10. Stamper RL, Lieberman MF, Drake MV. Carbonic anhydrase inhibitors [Internet]. *Becker-Shaffer’s Diagnosis and Therapy of the Glaucomas*. 2009. p. 407–19. Available from: <http://dx.doi.org/10.1016/b978-0-323-02394-8.00026-7>
11. Becker B. Decrease in intraocular pressure in man by a carbonic anhydrase inhibitor, diamox; a preliminary report. *Am J Ophthalmol*. 1954 Jan;37(1):13–5.
12. Lusthaus J, Goldberg I. Current management of glaucoma. *Med J Aust*. 2019 Mar;210(4):180–7.
13. Elvidge AR, Branch CL, Thompson GB. Observations in a Case of Hydrocephalus Treated with Diamox [Internet]. Vol. 14, *Journal of Neurosurgery*. 1957. p. 628–39. Available from: <http://dx.doi.org/10.3171/jns.1957.14.6.0628>
14. Rubin RC, Henderson ES, Ommaya AK, Walker MD, Rall DP. The production of cerebrospinal fluid in man and its modification by acetazolamide. *J Neurosurg*. 1966 Oct;25(4):430–6.
15. Supuran CT. Acetazolamide for the treatment of idiopathic intracranial hypertension. *Expert Rev Neurother*. 2015 Jul 7;15(8):851–6.
16. Kattah JC, Pula JH, Mejico LJ, McDermott MP, Kupersmith MJ, Wall M. CSF pressure, papilledema grade, and response to acetazolamide in the Idiopathic Intracranial Hypertension Treatment Trial. *J Neurol*. 2015 Oct;262(10):2271–4.
17. Acetazolamide in glaucoma. *Br Med J*. 1955 Aug 20;2(4937):479–81.
18. Becker B. Diamox and the therapy of glaucoma. *Am J Ophthalmol*. 1954 Jul;38(11):109–11.

19. Friedland BR, Mallonee J, Anderson DR. Short-term dose response characteristics of acetazolamide in man. *Arch Ophthalmol*. 1977 Oct;95(10):1809–12.
20. Alm A, Berggren L, Hartvig P, Roosdorp M. Monitoring acetazolamide treatment. *Acta Ophthalmol*. 1982 Feb;60(1):24–34.
21. Maus TL, Larsson LI, McLaren JW, Brubaker RF. Comparison of dorzolamide and acetazolamide as suppressors of aqueous humor flow in humans. *Arch Ophthalmol*. 1997 Jan;115(1):45–9.
22. Kass MA, Korey M, Gordon M, Becker B. Timolol and acetazolamide. A study of concurrent administration. *Arch Ophthalmol*. 1982 Jun;100(6):941–2.
23. Friedman DI, McDermott MP, Kieburz K, Kupersmith M, Stoutenburg A, Keltner JL, et al. The idiopathic intracranial hypertension treatment trial: design considerations and methods. *J Neuroophthalmol*. 2014 Jun;34(2):107–17.
24. Chaaban MR, Illing E, Riley KO, Woodworth BA. Acetazolamide for high intracranial pressure cerebrospinal fluid leaks. *Int Forum Allergy Rhinol*. 2013 Sep;3(9):718–21.
25. Bershad EM, Urfy MZ, Pechacek A, McGrath M, Calvillo E, Horton NJ, et al. Intracranial pressure modulates distortion product otoacoustic emissions: a proof-of-principle study. *Neurosurgery*. 2014 Oct;75(4):445–54; discussion 454–5.
26. Büki B, Chomiczki A, Dordain M, Lemaire JJ, Wit HP, Chazal J, et al. Middle-ear influence on otoacoustic emissions. II: contributions of posture and intracranial pressure. *Hear Res*. 2000 Feb;140(1-2):202–11.
27. Voss SE, Adegoke MF, Horton NJ, Sheth KN, Rosand J, Shera CA. Posture systematically alters ear-canal reflectance and DPOAE properties. *Hear Res*. 2010 May;263(1-2):43–51.
28. Williams MA, Malm J, Eklund A, Horton NJ, Voss SE. Distortion Product Otoacoustic Emissions and Intracranial Pressure During CSF Infusion Testing. *Aerosp Med Hum Perform*. 2016;87(10):844–51.
29. Heeg GP, Blanksma LJ, Hardus PLLJ, Jansonius NM. The Groningen Longitudinal Glaucoma Study. I. Baseline sensitivity and specificity of the frequency doubling perimeter and the GDx nerve fibre analyser. *Acta Ophthalmol Scand*. 2005 Feb;83(1):46–52.
30. Avan P, Giraudet F, Chauveau B, Gilain L, Mom T. Unstable distortion-product otoacoustic emission phase in Menière's disease [Internet]. Vol. 277, *Hearing Research*. 2011. p. 88–95. Available from: <http://dx.doi.org/10.1016/j.heares.2011.03.006>
31. Zinkernagel MS, Ebner A. Acetazolamide influences ocular pulse amplitude. *J Ocul Pharmacol Ther*. 2009 Apr;25(2):141–4.
32. Macdonald MJ, Gore SM, Cullen PM, Phillips CI. Comparison of ocular hypotensive effects of acetazolamide and atenolol. *Br J Ophthalmol*. 1977 May;61(5):345–8.
33. van Gestel A, Severens JL, Webers CAB, Beckers HJM, Jansonius NM, Schouten JSAG. Modeling complex treatment strategies: construction and validation of a discrete event simulation model for glaucoma. *Value Health*. 2010 Jun;13(4):358–67.
34. Katavisto M. The Diurnal Variations of Ocular Tension In Glaucoma. *Acta Ophthalmol Suppl*. 1964;SUPPL 78:1–130.
35. David R, Zangwill L, Briscoe D, Dagan M, Yagev R, Yassur Y. Diurnal intraocular pressure variations: an analysis of 690 diurnal curves. *Br J Ophthalmol*. 1992 May;76(5):280–3.

VASCULAR THEORY

5

Associations between tinnitus and glaucoma suggest a common mechanism: a clinical and population-based study

Allison R. Loiselle, Anna Neustaeter, Emile de Kleine, Pim van Dijk, Nomdo M. Jansonius

(Published: Hearing Research. February 2020; 386:107862)

ABSTRACT

The purpose of this study was to determine if there is an association between tinnitus and glaucoma. We tested this by first completing a clinic-based cross-sectional questionnaire study in which we sent a series of tinnitus-related questions to glaucoma patients and healthy subjects, and then followed up with a large population-based cross-sectional study in which glaucoma and tinnitus were also assessed by questionnaire. For the clinical study, we received 209 responses from glaucoma patients and 109 responses from healthy subjects (primarily the spouses of the patients). For the population-based study, we evaluated 79,866 participants. Logistic regression models were used to test the relationship between glaucoma and tinnitus; the clinical study analysis was adjusted for age, gender, BMI, hypertension, and diabetes and the population-based study was adjusted for these same variables with the addition of socioeconomic status and subjective hearing loss. For the clinical study, glaucoma patients had an 85% increase in odds for tinnitus (adjusted OR 1.85, 95% CI 1.10 to 3.05). The effect did not depend on pretreatment intraocular pressure, and the associated symptoms were not pulsatile in nature. For the population-based study, glaucoma patients had a 19% increase in odds for tinnitus (adjusted OR 1.19, 95% CI 1.02 to 1.40). Overall, our results suggest that those with glaucoma are more likely to have tinnitus than those without glaucoma. These results provide hypotheses for a mechanism involved in both tinnitus and glaucoma. One possible mechanism could be vascular dysregulation due to impairment of nitric oxide production.

5.1 INTRODUCTION

Primary open angle glaucoma (POAG) is a chronic and progressive eye disease that is characterized by damage to the retinal ganglion cells and their axons and subsequent loss of visual function. If left untreated, it can eventually lead to blindness. Although POAG is often associated with a high intraocular pressure (IOP), a large proportion of patients will have a normal eye pressure. The underlying cause of POAG is therefore still not well understood (1). There is a theory that, at least for some patients, glaucoma is an ocular manifestation of a wider problem of vascular dysregulation (2,3). Put simply, this means that the blood supply is not responsive to the shifting needs of various tissues. In this case, it is possible that organs other than the eye could be affected by insufficient blood flow as well.

In a previous study in our lab in which measurements of otoacoustic emissions were done in glaucoma patients (4), several subjects complained of tinnitus symptoms or wondered if their tinnitus would affect the test results. This anecdotal evidence led to a search of the literature, which yielded two articles that mentioned both glaucoma and tinnitus in the context of vascular dysregulation (5,6). At least one possible disease mechanism associated with vascular dysregulation that is associated with both glaucoma and tinnitus is a decrease in bioavailability of nitric oxide (NO; see section 4). A third, recent article actually connected glaucoma and tinnitus (7); this questionnaire study showed that tinnitus occurred significantly more often in glaucoma patients with a normal eye pressure. To our knowledge there are no reports confirming the finding that tinnitus is more prevalent in glaucoma.

The aim of this study was therefore to determine if there is an association between glaucoma and tinnitus. We tested this by (1) performing a clinical cross-sectional questionnaire study with glaucoma patients and healthy subjects and (2) comparing this finding with the association between glaucoma and tinnitus in a large, population-based cross-sectional Dutch cohort study (Lifelines).

5.2 METHODS

5.2.1 Clinical study population

The questionnaire (see section 3, original Dutch questions with translations) was sent to 371 patients with glaucoma, selected from the Groningen Longitudinal Glaucoma Study database (8). The main tinnitus question was: “Do you hear a ringing or rushing sound in your ear?”. In order to be eligible for the study, subjects had to be over 50 years of age, and have diagnosed glaucoma (as defined by Heeg et al (8)). We sent two questionnaires to each patient, and the patient was asked to fill in the first and give the second to a friend or partner without glaucoma. As a result, the healthy subjects in our study were primarily the spouses of the glaucoma patients. Patients and healthy subjects were explicitly asked to complete the questionnaire independently. All healthy subjects confirmed that they had no family history of glaucoma and were not under regular supervision of an ophthalmologist. In this way, we assured a glaucoma prevalence below 1% in the healthy (9). The ethics board of the

University Medical Center Groningen approved the study protocol. All patients provided written informed consent allowing data from their hospital files, such as IOP and visual field results, to be viewed. The study followed the tenets of the Declaration of Helsinki.

5.2.2 *Lifelines study population*

Lifelines is a prospective, population-based cohort study based in the Northern Netherlands (10). Lifelines is a multidisciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviours of 167,729 persons living in the north of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. The cohort is fully described elsewhere. In brief, recruitment was done through self-recruitment or general practitioner between the years of 2006-2013, and the approximately 167,000 participants will be followed for at least 30 years, and regularly receive questionnaires on a range of topics (11). One such questionnaire, the National Eye Institute Visual Functioning Questionnaire (NEI-VFQ-25), contains questions related to vision; we added questions regarding the presence of certain eye diseases as well as surgical, laser, or medical interventions for eye diseases; targeting age-related macular degeneration, cataract, diabetic retinopathy, dry eye disease, high eye pressure or glaucoma, refraction, and retinal detachment. The results from the first available 79,866 participants who completed the questionnaire were used in this study. We then investigated how this subsample responded to a different questionnaire in which questions about hearing and tinnitus were posed, specifically, the participants were asked “Do you ever hear a ringing or rushing sound in your ear(s)?” (Original in Dutch: “Hooort u suizen of fluiten in uw oor/oren?”). Response options were “never”, “sometimes”, and “always”.

The glaucoma definition (proxy) was questionnaire based. Since glaucoma is often undiagnosed, and ocular hypertension (high IOP without damage to the visual system) is sometimes mixed-up with glaucoma, we combined self-reported glaucoma with the presence of glaucoma-specific visual complaints, in order to define glaucoma as accurately as possible with a questionnaire. For the assessment of visual complaints, we used the NEI-VFQ-25. Definite glaucoma cases were those who had a history of glaucoma surgery. Their visual complaint pattern was compared to that of healthy subjects, yielding the glaucoma-specific visual complaints (mainly complaints regarding peripheral vision and visual functioning in low luminance). Subsequently, probable glaucoma cases were those who self-reported glaucoma (without surgery) and displayed glaucoma-specific visual complaints. Possible glaucoma cases were those who self-reported glaucoma (without surgery) or displayed glaucoma-specific visual complaints, but not both. Unaffected were those who neither self-reported glaucoma nor displayed glaucoma specific visual complaints. Details have been fully described elsewhere.(12) For this study, glaucoma classification was binarized, where ‘definites’ and ‘probables’ were categorized as affected, the ‘unaffected’ was the same, and those classified as ‘possible’ were excluded.

5.2.3 Case-control data analysis

Patients and controls were described with mean and standard deviation (SD) for normally distributed variables; means were compared using the Student's t-test. Proportions for demographics and for the questionnaire results were compared using a chi-square test. For the patients, disease severity was quantified as the standard automated perimetry (Humphrey field analyzer 30-2 SITA; Carl Zeiss Meditec, Jena, Germany) mean deviation (MD) of the worse eye.

A logistic regression was performed using a model with tinnitus as the outcome variable and glaucoma, age, gender, body mass index (BMI; categorical variable with "low" ≤ 18.5 , "normal" = 18.6 - 24.9, "high" ≥ 25 kg/m²), hypertension (antihypertensive medication use), and diabetes (use of Metformin or insulin) as explanatory variables. BMI, hypertension and diabetes were added to the model as these have both previously been associated with tinnitus (1,13–17) and glaucoma (1). This was repeated after stratifying glaucoma into POAG and NTG, using a pre-treatment IOP > 21 mmHg as cut-off. An odds ratio (OR) and 95% confidence interval (CI) were calculated to identify the likelihood of tinnitus when glaucoma was present.

5.2.4 Lifelines data analysis

A logistic regression was also performed for the Lifelines cohort with tinnitus as the outcome variable and glaucoma, age, gender, categorical BMI, hypertension, diabetes, socioeconomic status (SES), and subjective hearing loss as explanatory variables. SES and hearing loss data were available for Lifelines and were included in the model because both are associated with tinnitus (18,19). SES is possibly also related to glaucoma (20,21), while the relationship between hearing loss and glaucoma is not clear for all glaucoma types (22). For this analysis, tinnitus was binarized (never = no; sometimes or always = yes). Hypertension was defined as one of the following: systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of antihypertensives (ATC code C02), diuretics (ATC code C03), ACE inhibitors (ATC code C09) beta-blockers (ATC code C07), or calcium channel blockers (ATC code C08). Diabetes was defined as one of the following: 'yes' to the question 'Do you have diabetes mellitus?', or use of metformin (ATC code A10BA or A10BD) or insulin (ATC code A10A). Socioeconomic status was defined by highest educational level achieved, as per van Zon et al. (23). Socioeconomic status was categorized into low, medium, high, and other (a text box where participants could fill in their own answer; in this study the responses were primarily vocational school). Hearing loss was defined using the question: "Are you limited by problems with your hearing in daily life?" (Original question in Dutch: "Wordt u in het dagelijks leven beperkt door problemen met uw gehoor?") and answer options were "not at all", "somewhat", "severely". An OR and 95% CI were calculated in order to identify the likelihood of tinnitus when glaucoma was present and to compare the Lifelines data with the case-control questionnaire.

All analyses were performed using R (version 3.3.3; R Foundation for Statistical Computing, Vienna, Austria). A P-value of 0.05 or less was considered statistically significant.

5.3 RESULTS

For the case-control study, we sent 371 questionnaires and received 209 responses from glaucoma patients (response rate 56.3%) and 109 from controls (the actual response rate is not known, as we don't know how many patients gave the second questionnaire to a spouse or friend - see section 5.2.1). From the Lifelines cohort we included 390 participants as "affected" and 72,319 controls. Table 5.1 shows the characteristics and demographics of both study populations. For the case-control study, glaucoma patients compared with controls were similar in age and gender. The average age of participants within the Lifelines cohort was approximately 50, that is, significantly different from those that are affected with glaucoma (62 years, on average).

Table 5.1 | Characteristics of the study populations

	Case-control Study		
	Glaucoma n=209	Healthy n=109	P-value
Age (years; mean[SD])	72.6 [8.7]	70.9 [7.5]	0.07
Gender (% female)	51.2	56.9	0.33
Pre-treatment IOP (mmHg; median[IQR])	26.0 [16.0 to 36.0]	NA	NA
MD (dB; median[IQR])	-9.7 [-22.9 to -3.5]	NA	NA
	Lifelines Study		
	Glaucoma n=390	Healthy n=72,319	P-value
Age (years; mean[SD])	62.1 [11.7]	49.6 [12.3]	<0.001
Gender (% female)	60.3	59.1	0.69

SD = standard deviation; *IOP* = intraocular pressure; *IQR* = interquartile range; *MD* = standard automated perimetry mean deviation.

Table 5.2 presents the original questions and results from the case-control tinnitus questionnaire. Glaucoma patients were more likely to have tinnitus (Q1; $P=0.01$), but, once present, the frequency of symptoms (Q6; $P=0.47$) and the prevalence of pulsatile tinnitus characteristics (Q3; $P=0.70$) did not differ between groups. Only eight subjects had both unilateral glaucoma and unilateral tinnitus. In 3 out of these 8, glaucoma and tinnitus were on the same side, indicating no clear ipsilateral relationship.

Table 5.2 | Tinnitus questionnaire for case-control study (with the original questions in Dutch)

Questions	Glaucoma (n=209)	Controls (n=109)	P-value
Q1. Do you ever hear a ringing or rushing sound in your ear? (Q1. Hoort u ooit een suizen of fluitend geluid in uw oor?)	46.4% yes	31.2% yes	0.01
If yes on Q1	(n=97)	(n=34)	
Q2. If so, in which ear? (Q2. Zo ja, in welk oor?)	16.5% right 16.5% left 67.0% both	20.6% right 20.6% left 58.8% both	0.69
Q3. Does it pulsate? (Like the sound of your heart beat?) (Q3. Pulseert het? (Zoals het geluid van uw hartslag?))	16.5% yes	11.7% yes	0.70
Q4. Does it get quicker if you do exercise? (Q4. Wordt het sneller als u aan lichaamsbeweging doet?)	12.4% yes	2.9% yes	0.17
Q5. Does it get worse if you change body positions? (Q5. Wordt het erger als u van houding wisselt?)	3.1% yes	0% yes	0.53
Q6. How often do you hear it? (Q6. Hoe vaak hoort u het?)	8.2% rarely 53.6% sometimes 38.1% always	5.9% rarely 44.1% sometimes 50.0% always	0.47

In the case-control study, after controlling for age, gender, BMI, hypertension, and diabetes, patients with glaucoma had an 85% increase in odds for glaucoma compared to those without tinnitus (binarized using Q1; adjusted OR 1.85; 95% CI 1.10 to 3.05; $P=0.018$). Table 5.3 shows the multivariable logistic regression model. There was no gender-specific effect of glaucoma (interaction term gender*glaucoma not significant; $P=0.29$). When analyzing the glaucoma subgroups NTG (48 patients) and POAG (161 patients) separately, the relationship between NTG and tinnitus (OR 1.77; 95% CI 0.85 to 3.69; $P=0.12$) appeared to be similar to between POAG and tinnitus (OR 1.85; 95% CI 1.09 to 3.15; $P<0.01$) but did not reach significance, possibly related to the relatively small number of NTG patients. The standard automated perimetry MD value (worse eye) did not differ between these subgroups (median [IQR] for POAG -9 (-23 to -5) dB and for NTG -10 (-18 to -2) dB; $P=0.84$), indicating equal glaucoma severity amongst both subgroups.

Table 5.3 | Logistic regression models for the case-control and Lifelines studies with tinnitus as outcome variable

	Case-Control Study		Lifelines Study	
	Adjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Glaucoma (yes)	1.85 (1.10, 3.05)	0.018	1.19 (1.02, 1.40)	0.028
Age (yrs)	0.99 (0.96, 1.02)	0.59	1.01 (1.01, 1.02)	<0.001
Gender (female)	0.40 (0.25, 0.65)	< 0.001	0.83 (0.81-0.85)	<0.001
BMI (reference: normal)		0.61	1.06 (0.90, 1.23)	0.042
Underweight	1.34 (0.44, 4.10)		0.97 (0.95, 0.99)	
Overweight	0.69 (0.41, 1.13)			
Hypertension (yes)	1.30 (0.77, 2.18)	0.32	1.09 (1.05, 1.12)	<0.001
Diabetes (yes)	0.94 (0.38, 2.33)	0.89	1.06 (0.99, 1.14)	0.087
SES (reference: high)			1.42 (1.37, 1.47)	<0.001
Low	NA	NA	1.20 (1.16, 1.23)	
Medium			1.35 (1.25, 1.47)	
Other				
Hearing loss	NA	NA	3.52 (3.41, 3.65)	<0.001
(reference: not at all)			6.13 (5.16, 7.28)	
Somewhat				
Severely				

BMI = Body mass index, SES = Socioeconomic Status

In Lifelines, the logistic regression showed that, when controlled for age, gender, BMI, hypertension, diabetes, socioeconomic status, and hearing loss, glaucoma patients had a 19% increase in odds for tinnitus compared to those without glaucoma (adjusted OR 1.19; 95% CI 1.02 to 1.40; $P=0.028$). Table 5.3 shows the results from the logistic regression model. Again, there was no gender-specific effect of glaucoma (interaction term gender*glaucoma not significant; $P=0.72$). Figure 5.1 shows a comparison between the frequency of symptoms in the case-control study and Lifelines.

FIG 5.1 Frequency of tinnitus complaints. A comparison between the frequency of tinnitus complaints in the case-control study (left panel) and Lifelines study (right panel). In Lifelines there was no option to choose "rarely".

5.4 DISCUSSION

In the current study we find that in both the case-control and Lifelines studies, those with glaucoma are more likely to have tinnitus than those without glaucoma. The symptoms are not pulsatile in nature, and there is no gender-specific effect of glaucoma. Normal tension glaucoma and primary open angle glaucoma patients seem to be affected equally.

There has only been one previous study investigating the association between glaucoma and tinnitus, in which questionnaires from 246 NTG patients and 1,116 controls were analyzed and showed NTG patients were more likely to have tinnitus (7). This is in agreement with our findings. In our study, however, we found this association for all glaucoma patients, not just the NTG subset.

We know that tinnitus is associated with hearing loss (19), so an obvious question is: Do glaucoma patients simply have more hearing loss than healthy people? There has been research that demonstrates a relationship between pseudoexfoliative glaucoma (PEX) and hearing loss, however this result is not found in POAG (22,24–26), and we do not include PEX patients in our study. Hearing loss is more common with older age, but in the case-control study, the groups were similar in age and the analyses were adjusted for age in both the case-control and Lifelines studies. Additionally, in the Lifelines study we controlled for subjective hearing loss by including a question about the impact of hearing loss on daily life, and the relationship between glaucoma and tinnitus was still significant.

While it is possible that vascular dysregulation may play a role in both tinnitus and glaucoma, it is a broad term and could result from a multitude of causes. One possible specific cause of the relationship between glaucoma and tinnitus could be the inhibition of nitric oxide (NO) production. Interestingly, NO is also reduced in the jugular vein of patients with tinnitus, which may lead to disruption of the microcirculation in the ear (27). It was also shown, in an animal model, that after noise exposure the modulation of NO producing enzymes in the cochlear nucleus determined whether or not the animal developed tinnitus (28). It is known that *CAVI*, *CAV2*, and *NOS3* — genes that relate to NO production — can be affected in glaucoma (29–32). These genes are crucial to the L-Arginine-NO pathway, and have been shown to alter ocular blood flow (33). If this pathway is inhibited due to a deleterious change in the aforementioned genes, as in glaucoma, the body can utilize an alternative method whereby nitrates from the diet are converted to nitrites by commensal bacteria in the saliva, and then converted to NO in the stomach (the Nitrate-nitrite-NO pathway) (34,35). Interestingly, it has already been shown that high levels of nitrates in the diet can reduce the risk of glaucoma by 20-30% (36). The Nitrate-nitrite-NO pathway may therefore be extremely important in glaucoma. However, in order to convert nitrites to NO in the stomach, a normal gastric pH must be present, therefore the use of any medications that raise the pH in the stomach, like proton pump inhibitors (PPIs) could hinder this pathway as well (37). In our case-control study, those taking PPIs had a 2.35 fold increase in odds for glaucoma (OR 2.35; 95% CI 1.26 to 4.39; $p < 0.01$). The theory that NO could connect both tinnitus and glaucoma needs to be confirmed.

There were some limitations in this study. We relied on self-reporting of tinnitus and not clinical evaluation in both the case-control and Lifelines data sets. However, the questions were phrased simply, and tinnitus is subjective by nature so there is no reason to suspect under or over-reporting in either group. Additionally, selective responses from patients who had tinnitus seem unlikely because the tinnitus questions in both the case-control and Lifelines studies were part of larger questionnaires on many different topics. Second, while in the case-control study we had a strict definition of POAG and NTG, in the Lifelines data we used a proxy for glaucoma. By using additional questions, secondary glaucoma was excluded as much as possible, but narrow-angle glaucoma could still confound the data. However, in a previous population-based Dutch cohort study where they did a thorough eye exam in all participants, it was shown that the majority of cases were POAG or NTG, with secondary or narrow-angle glaucoma as exceptions (38). The inclusion of ‘probable’ glaucoma cases in the proxy could mean that some subjects included as patients were actually healthy. Consequently, the difference between the patient and control group reported here presumably reflects a lower boundary to the actual relationship between tinnitus and glaucoma. One strong point is the high number of participants included in the Lifelines study. Additionally, the agreement between the case-control and Lifelines studies demonstrates that the association between tinnitus and glaucoma is a repeatable finding.

5.5 CONCLUSION

Patients with glaucoma are more likely to have tinnitus than those without glaucoma. Future research should focus on the possibility that vascular dysregulation due to impairment of NO production — from both genetic expression and diet — could potentially lead to both of these diseases. Their chronology should also be characterized. Do they occur simultaneously? Or does one come before the other? If the latter is true, then one disease may be a predictor of the other. Further research could also explore the relationships between glaucoma, tinnitus, and other diseases that have been associated with aberrant NO behavior, specifically type II diabetes (39,40), dementia (41,42), and sleep apnea (43,44). Investigation of these relationships could lead to an improvement in quality of life for the patients and a better understanding of the pathophysiology of both glaucoma and tinnitus.

REFERENCES

1. Janssen SF, Gorgels TGMF, Ramdas WD, Klaver CCW, van Duijn CM, Jansonius NM, et al. The vast complexity of primary open angle glaucoma: disease genes, risks, molecular mechanisms and pathobiology. *Prog Retin Eye Res.* 2013 Nov;37:31–67.
2. Harris A, Wirostko B. Cerebral Blood Flow in Glaucoma Patients. *J Glaucoma.* 2013;22:S46–8.
3. Pasquale LR. Vascular and autonomic dysregulation in primary open-angle glaucoma. *Curr Opin Ophthalmol.* 2016 Mar;27(2):94–101.
4. Loissele AR, de Kleine E, van Dijk P, Jansonius NM. Noninvasive intracranial pressure assessment using otoacoustic emissions: An application in glaucoma. *PLoS One.* 2018 Oct 1;13(10):e0204939.
5. Konieczka K, Fränkl S, Todorova MG, Henrich PB. Unstable oxygen supply and glaucoma. *Klin Monbl Augenheilkd.* 2014 Feb;231(2):121–6.
6. Flammer J, Konieczka K, Flammer AJ. The primary vascular dysregulation syndrome: implications for eye diseases. *EPMA J.* 2013 Jun 7;4(1):14.
7. Konieczka K, Choi HJ, Koch S, Fankhauser F, Schoetzau A, Kim DM. Relationship between normal tension glaucoma and Flammer syndrome. *EPMA J.* 2017 Jun;8(2):111–7.
8. Heeg GP, Blanksma LJ, Hardus PLLJ, Jansonius NM. The Groningen Longitudinal Glaucoma Study. I. Baseline sensitivity and specificity of the frequency doubling perimeter and the GDx nerve fibre analyser. *Acta Ophthalmol Scand.* 2005 Feb;83(1):46–52.
9. Wolfs RC, Borger PH, Ramrattan RS, Klaver CC, Hulsman CA, Hofman A, et al. Changing views on open-angle glaucoma: definitions and prevalences--The Rotterdam Study. *Invest Ophthalmol Vis Sci.* 2000 Oct;41(11):3309–21.
10. Klijs B, Scholtens S, Mandemakers JJ, Snieder H, Stolk RP, Smidt N. Representativeness of the LifeLines Cohort Study. *PLoS One.* 2015;10(9):e0137203.
11. Scholtens S, Smidt N, Swertz MA, Bakker SJL, Dotinga A, Vonk JM, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol.* 2015 Aug;44(4):1172–80.
12. Neustaeter A, Vehof J, Snieder H, Jansonius NM. Glaucoma in large-scale population-based epidemiology: a questionnaire-based proxy [Internet]. *Eye.* 2020. Available from: <http://dx.doi.org/10.1038/s41433-020-0882-4>
13. Figueiredo RR, Azevedo AA, Penido NDO. Positive Association between Tinnitus and Arterial Hypertension. *Front Neurol.* 2016 Oct 5;7:171.
14. Gibrin PCD, Melo JJ, Marchiori LL de M. Prevalence of tinnitus complaints and probable association with hearing loss, diabetes mellitus and hypertension in elderly. *Codas.* 2013;25(2):176–80.
15. Shargorodsky J, Curhan GC, Farwell WR. Prevalence and characteristics of tinnitus among US adults. *Am J Med.* 2010 Aug;123(8):711–8.
16. Lavinsky L, Oliveira MW, Bassanesi HJC, D'Avila C, Lavinsky M. Hyperinsulinemia and tinnitus: a historical cohort. *Int Tinnitus J.* 2004;10(1):24–30.
17. Lee D-H, Kim YS, Chae HS, Han K. Nationwide analysis of the relationships between mental health, body mass index and tinnitus in premenopausal female adults in Korea: 2010-2012 KNHANES. *Sci Rep.* 2018 May 4;8(1):7028.

18. Hoekstra CEL, Wesdorp FM, van Zanten GA. Socio-demographic, health, and tinnitus related variables affecting tinnitus severity. *Ear Hear.* 2014 Sep;35(5):544–54.
19. Eggermont JJ. *The Neuroscience of Tinnitus.* 2012.
20. Ramdas WD, Wolfs RCW, Hofman A, de Jong PTVM, Vingerling JR, Jansonius NM. Lifestyle and risk of developing open-angle glaucoma: the Rotterdam study. *Arch Ophthalmol.* 2011 Jun;129(6):767–72.
21. Oh SA, Ra H, Jee D. Socioeconomic Status and Glaucoma: Associations in High Levels of Income and Education. *Curr Eye Res.* 2019 Apr;44(4):436–41.
22. Tryggvason G, Jonasson F, Cotch MF, Li C-M, Hoffman HJ, Themann CL, et al. Hearing in older adults with exfoliation syndrome/exfoliation glaucoma or primary open-angle glaucoma. *Acta Ophthalmol.* 2016 Mar;94(2):140–6.
23. van Zon SKR, Snieder H, Bültmann U, Reijneveld SA. The interaction of socioeconomic position and type 2 diabetes mellitus family history: a cross-sectional analysis of the Lifelines Cohort and Biobank Study [Internet]. Vol. 7, *BMJ Open.* 2017. p. e015275. Available from: <http://dx.doi.org/10.1136/bmjopen-2016-015275>
24. Shapiro A, Siglock TJ, Ritch R, Malinoff R. Lack of association between hearing loss and glaucoma. *Am J Otol.* 1997 Mar;18(2):172–4.
25. Paliobei VP, Psillas GK, Mikropoulos DG, Haidich A-B, Constantinidis J, Konstas AGP. Hearing Evaluation in Patients with Exfoliative and Primary Open-Angle Glaucoma. *Otolaryngol Head Neck Surg.* 2011 Jul;145(1):125–30.
26. Hayreh SS, Podhajsky P, Zimmerman MB. Ocular and optic nerve head ischemic disorders and hearing loss. *Am J Ophthalmol.* 1999 Nov;128(5):606–11.
27. Neri S, Signorelli S, Pulvirenti D, Mauceri B, Cilio D, Bordonaro F, et al. Oxidative stress, nitric oxide, endothelial dysfunction and tinnitus. *Free Radic Res.* 2006 Jun;40(6):615–8.
28. Coomber B, Kowalkowski VL, Berger JI, Palmer AR, Wallace MN. Modulating central gain in tinnitus: changes in nitric oxide synthase in the ventral cochlear nucleus. *Front Neurol.* 2015 Mar 10;6:53.
29. Emam WA, Zidan HE, Abdulhalim B-EH, Dabour SA, Ghali MA, Kamal AT. Endothelial nitric oxide synthase polymorphisms and susceptibility to high-tension primary open-angle glaucoma in an Egyptian cohort. *Mol Vis.* 2014 Jun 12;20:804–11.
30. Kang JH, Wiggs JL, Rosner BA, Hankinson SE, Abdrabou W, Fan BJ, et al. Endothelial nitric oxide synthase gene variants and primary open-angle glaucoma: interactions with sex and postmenopausal hormone use. *Invest Ophthalmol Vis Sci.* 2010 Feb;51(2):971–9.
31. Thorleifsson G, Walters GB, Hewitt AW, Masson G, Helgason A, DeWan A, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. *Nat Genet.* 2010 Oct;42(10):906–9.
32. Logan JEJ, Chakravarthy U, Hughes AE, Patterson CC, Jackson JA, Rankin SJA. Evidence for Association of Endothelial Nitric Oxide Synthase Gene in Subjects with Glaucoma and a History of Migraine. *Investigative Ophthalmology & Visual Science.* 2005;46(9):3221.
33. Schmetterer L, Polak K. Role of nitric oxide in the control of ocular blood flow. *Prog Retin Eye Res.* 2001 Nov;20(6):823–47.
34. Weitzberg E, Hezel M, Lundberg JO. Nitrate-nitrite-nitric oxide pathway:

-
- implications for anesthesiology and intensive care. *Anesthesiology*. 2010 Dec;113(6):1460–75.
35. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov*. 2008;7(2):156–67.
 36. Kang JH, Willett WC, Rosner BA, Buys E, Wiggs JL, Pasquale LR. Association of Dietary Nitrate Intake With Primary Open-Angle Glaucoma: A Prospective Analysis From the Nurses' Health Study and Health Professionals Follow-up Study. *JAMA Ophthalmol*. 2016 Mar;134(3):294–303.
 37. Lundberg JO, Weitzberg E, Lundberg JM, Alving K. Intra gastric nitric oxide production in humans: measurements in expelled air. *Gut*. 1994 Nov;35(11):1543–6.
 38. Skenduli-Bala E, de Voogd S, Wolfs RCW, van Leeuwen R, Ikram MK, Jonas JB, et al. Causes of incident visual field loss in a general elderly population: the Rotterdam study. *Arch Ophthalmol*. 2005 Feb;123(2):233–8.
 39. Tessari P, Cecchet D, Cosma A, Vettore M, Coracina A, Millioni R, et al. Nitric oxide synthesis is reduced in subjects with type 2 diabetes and nephropathy. *Diabetes*. 2010 Sep;59(9):2152–9.
 40. Assmann TS, Brondani LA, Bouças AP, Rheinheimer J, de Souza BM, Canani LH, et al. Nitric oxide levels in patients with diabetes mellitus: A systematic review and meta-analysis. *Nitric Oxide*. 2016 Dec 30;61:1–9.
 41. Katusic ZS, Austin SA. Neurovascular Protective Function of Endothelial Nitric Oxide - Recent Advances. *Circ J*. 2016 Jun 24;80(7):1499–503.
 42. Austin SA, Santhanam AV, Hinton DJ, Choi D-S, Katusic ZS. Endothelial nitric oxide deficiency promotes Alzheimer's disease pathology. *J Neurochem*. 2013 Dec;127(5):691–700.
 43. Weiss JW, Woodrow Weiss J, Liu Y, Li X, Ji E-S. Nitric oxide and obstructive sleep apnea. *Respir Physiol Neurobiol*. 2012;184(2):192–6.
 44. Badran M, Golbidi S, Ayas N, Laher I. Nitric Oxide Bioavailability in Obstructive Sleep Apnea: Interplay of Asymmetric Dimethylarginine and Free Radicals. *Sleep Disord*. 2015 May 6;2015:387801.
-

6

Nitric oxide bioavailability and excitotoxicity in glaucoma and tinnitus

*Allison R. Loiselle, Aruna Rikkers, Emile de Kleine,
Pim van Dijk, Nomdo M. Jansonius*

ABSTRACT

ow nitric oxide (NO) bioavailability and oxidative stress may be common mechanisms behind glaucoma and tinnitus. NO itself diffuses quickly and is difficult to measure, however, certain metabolites can be measured as biomarkers of NO production. The aim of this study was to test these biomarkers of NO in glaucoma patients and controls both with and without tinnitus. To do this, serum levels of L-arginine (ARG), L-ornithine (ORN), L-citrulline (CIT), L-glutamate (GLU), and L-glutamine (GLN) were assessed in 23 patients with primary open angle glaucoma (14 with tinnitus and 9 without) and 20 controls (13 with tinnitus and 7 without). The groups were age-similar. A quantile regression was performed for each biomarker with presence of glaucoma and presence of tinnitus as independent variables. There was no significant difference in NO biomarker concentrations between glaucoma and controls. Presence of tinnitus also did not significantly affect the biomarkers. However, there was a trend for increased ARG in glaucoma patients compared to controls, especially in those with tinnitus.

6.1 INTRODUCTION

Glaucoma is a chronic and progressive eye disease characterized by cupping of the optic disc, thinning of the retinal nerve fiber layer, loss of retinal ganglion cells, and loss of visual function. The pathophysiology of glaucoma is not yet fully understood. A high intraocular pressure (IOP) is currently the only modifiable risk factor for the disease. However, some glaucoma patients have normal IOP (normal tension glaucoma [NTG]). Altered ocular perfusion has been proposed as a possible mechanism in the development of glaucoma as well. (1) One of the main factors of retinal perfusion is nitric oxide (NO). (2) NO has a vasodilating effect, and research has found that NO markers are decreased in patients with glaucoma. (3,4) Genetics linked to the production of NO are also found to be altered in glaucoma patients. (5,6) However, the research on the relationship between plasma NO biomarkers and glaucoma is still scarce, and it is yet to be determined if aberrant NO production could explain the association between glaucoma and other diseases with a vascular component.

Previous research found that glaucoma patients are more likely to have tinnitus than healthy people. (7) Tinnitus has been linked to a decrease of NO in the jugular vein (8) and, just as with glaucoma patients, genetic alterations linked to the production of NO were correlated with tinnitus. (9) A decrease in NO bioavailability could be a common pathological mechanism between these two diseases.

It is difficult to analyze NO directly, as it has a biological half-life of only 3-30 seconds. (10) Therefore, an understanding of the production process of NO is needed. NO can be produced through different pathways. In normal physiological conditions, the L-arginine (ARG) pathway is the dominant one (Figure 6.1). In this pathway, ARG together with oxygen is synthesised into NO and L-citrulline (CIT) via an enzyme called nitric oxide synthase (NOS). There are a few ways in which this process can be defective. First, it is possible that those with decreased NO bioavailability have inherently low ARG levels. Second, NO production can be inhibited by asymmetric dimethylarginine (ADMA), an endogenous analogue of ARG. (11) Third, ARG can be preferentially synthesised into L-ornithine (ORN) and urea when arginase levels are high. As such, both low and high ARG levels can be associated with a shortage of NO.

The aim of this study was to determine if low NO and increased excitotoxicity are associated with glaucoma and if so, if the associations are influenced by the presence of tinnitus. For this purpose we performed a cross-sectional clinical study in which blood samples of primary open angle glaucoma (POAG) patients were analyzed for markers of impaired NO production, namely: arginine, ornithine, citrulline, glutamate, and glutamine. The plasma levels of these amino acids were then compared to those of age-similar controls. Both groups were also balanced for the presence of tinnitus.

FIG 6.1 In the normal NO production pathway, arginine is converted into NO and citrulline. High levels of ADMA can cause arginine to de-couple with NOS, leading to decreased NO production. High arginase levels lead to arginine selectively converting to ornithine and urea instead of NO. Additionally, if NOS is uncoupled due to low oxygen levels or a lack of BH₄ (tetrahydrobiopterin), reactive oxygen species (ROS) are produced and oxidative damage can occur.

FIG 6.2 Increased extracellular glutamate levels lead to an overexpression of NMDA receptors and an increase of calcium entering the cell. This then leads to prolonged depolarization of the cell, overactivation of NOS and therefore excitotoxicity.

6.2 METHODS

6.2.1 Study population

All patients in this study were selected from the Groningen Longitudinal Glaucoma study (GLGS) database.⁽¹⁾ Patients were required to have diagnosed POAG. Glaucoma was defined according to Heeg et al. (19) We required a reproducible (same hemifield and at least partially overlapping) visual field defect (Humphrey Field Analyzer 30–2 SITA fast; Carl Zeiss, Jena, Germany; criterion: ‘glaucoma hemifield test’ outside normal limits) in at least one eye that had to be compatible with glaucoma and without any other explanation. For POAG, the additional requirements were an open anterior chamber angle and no signs of secondary glaucoma.

We asked patients to give an information letter to a friend or partner without glaucoma. As a result, the healthy subjects in our study were primarily the spouses of the glaucoma patients. In order to be eligible to participate, controls needed an IOP of 21 mmHg or lower, no eye disease, and no family history of glaucoma (determined by a questionnaire). To exclude eye disease, we performed optical coherence tomography (OCT-HS100; Canon, Tokyo, Japan; considered normal if the mean retinal nerve fiber layer and retinal ganglion cell layer thickness in macular area were above the 5th percentile) and frequency doubling technology (FDT; Carl Zeiss, Jena, Germany; no reproducibly abnormal test locations allowed in C20-1 screening mode).

The groups were balanced for age, gender, and the presence of tinnitus, as defined previously.⁽⁷⁾ The ethics board of the University Medical Center Groningen approved the study protocol. All patients provided written informed consent allowing data from their hospital files, such as IOP and visual field results, to be viewed. The study followed the tenets of the Declaration of Helsinki.

6.2.2 Plasma sample testing

For the glaucoma patients, plasma samples were obtained following a regular visit to the outpatient department, either in the morning or in the afternoon. The majority of controls who participated (15 of 20) came with a partner who had glaucoma. Therefore, the majority of plasma samples were obtained at the same time for patient/control pairs. Subjects were not fasted.

Plasma was stored at -20°C for 1–5 days prior to analyses. Plasma concentrations were determined using a commercial method based on ion exchange chromatography with post column derivatization with Ninhydrin on a Biochrom 30+ or Biochrom 30 analyser (Pharmacia Biotech, Cambridge, UK). For sample preparation, we refer to van Vliet et al. (20) A physiological amino acid calibration standard was used for calibration (Sigma-Aldrich, Darmstadt, Germany). External quality control samples (ERNDIM) showed excellent accuracy, precision, and recovery.

6.2.3 Data analysis

The characteristics of the study population were described with medians and interquartile ranges (IQR). Comparisons between POAG patients and controls regarding the descriptive

statistics and the metabolites were made using Wilcoxon Ranked-Sum tests, while proportions were compared using a chi-square test. To determine if there was a difference in NO biomarker concentrations between POAG patients and controls with the addition of the influence of tinnitus, quantile regressions were performed with each NO biomarker as a dependent variable and presence of glaucoma and presence of tinnitus as independent variables.

All analyses were performed using R (Version 4.0.1; The R Foundation for Statistical Computing, Vienna, Austria). A P-value of 0.05 or less was considered statistically significant.

6.3 RESULTS

Table 6.1 shows the characteristics of the study population. There were no significant differences in demographic characteristics between POAG patients and controls. There was also no significant difference in the time of day the blood draws were performed.

Table 6.1 | Characteristics of the study population

Group	Glaucoma (n=23)	Controls (n=20)	P-value
Age	67.0	64.0	0.61
(years; median [IQR])	(63.0 to 71.0)	(58.9 to 69.1)	
Gender (% Female)	39.1%	40.0%	0.95
Tinnitus (% Yes)	39.1%	35.0%	0.78
IOP0 (mmHg; median [IQR])	29.5 (22.4 to 36.6)	NA	-
VF MD of better eye (dB; median [IQR])	-4.1 (-0.5 to -7.7)	NA	-
VF MD of worse eye (dB; median [IQR])	-14.8 (-7.5 to -22.1)	NA	-
Time of day for blood draw (% before 12:00)	39.1%	40.0%	0.95

IQR = interquartile range, IOP0 = intraocular pressure before onset of IOP lowering treatment, VF MD = standard automated perimetry mean deviation, NA = Not applicable.

Figure 6.3 shows the distribution of NO biomarker concentrations for patients and controls and their respective lab reference ranges. Figure 6.4 shows the ARG concentrations in glaucoma and controls separated by those with tinnitus and those without, suggesting a trend of increased ARG in the group with both diseases. Table 6.2 shows the concentrations of the NO biomarkers in POAG patients and controls and Table 6.3 shows the results of the quantile regression with the inclusion of presence of tinnitus as an independent variable. There were no significant differences in amino acid concentrations between patients and controls, and no apparent influence of tinnitus.

FIG 6.3 No biomarker concentrations ($\mu\text{mol/L}$) in patients and controls compared to the normal reference range provided by the lab (shaded regions). The scale for the y-axis is the same for all biomarkers except glutamine (GLN).

FIG 6.4 Arginine concentrations ($\mu\text{mol/L}$) in POAG patients and controls, separated by the presence or absence of tinnitus.

Table 6.2 | Nitric oxide biomarker concentrations (μmol/L)

Group	Glaucoma (n=23)	Controls (n=20)	P-value
Arginine (μmol/L; median[IQR])	68.0 (59.7 to 76.2)	54.5 (35.7 to 73.2)	0.14
Ornithine (μmol/L; median[IQR])	99.0 (91.0 to 107.0)	95.5 (84.0 to 107.0)	0.78
Citrulline (μmol/L; median[IQR])	30.0 (25.3 to 34.7)	31.0 (25.5 to 36.5)	0.77
Glutamate (μmol/L; median[IQR])	43.0 (29.7 to 56.2)	39.5 (9.0 to 70.0)	1.0
Glutamine (mol/L; median[IQR])	568.0 (526.5 to 609.5)	582.5 (552.0 to 613.0)	0.52

Table 6.3 | Quantile regression analysis for glaucoma and tinnitus with each NO biomarker as the dependent variable

	Arginine		Ornithine		Citrulline		Glutamate		Glutamine	
	Coef	P-value	Coef	P-value	Coef	P-value	Coef	P-value	Coef	P-value
Glaucoma (Yes)	13.0	0.15	5.0	0.46	-1.0	0.77	7.0	0.35	-14.0	0.54
Tinnitus(Yes)	4.0	0.59	7.0	0.24	0.0	1.0	9.0	0.29	16.0	0.49

6.4 DISCUSSION

In this study we find that biomarkers in glaucoma patients do not point toward a lower bioavailability of NO or higher excitotoxicity. Presence of tinnitus was also not associated with biomarker concentrations suggestive of NO production impairment.

The primary way NO is produced is through conversion of ARG and oxygen. ARG can be utilized in the retina through two different pathways. The first is through NOS, which produces NO and CIT. Inhibition of NOS in glaucoma could impair ocular hemodynamics through endothelial dysfunction. The inhibition of NOS has been shown to have less of an effect on optic nerve head blood flow and fundus pulsation amplitude in glaucoma patients compared to controls, suggesting an already altered NO production mechanism in glaucoma.(21) Inhibition of NOS has also been demonstrated through low nitrite (an end product of NO) in the aqueous humor of patients with glaucoma compared to controls.(3,4) Although this was not demonstrated in all studies.(22) One potential confounder in testing nitrite in aqueous humor is that measurements are completed during ocular surgeries. An isoform of NOS called inducible NOS (iNOS) is stimulated by transient ischemia and by inflammatory microenvironments, both of which could be present during ocular surgery. For this reason, plasma measurements may be beneficial. Our plasma NO biomarker measurements do not suggest NOS inhibition in glaucoma patients or those with tinnitus.

The second pathway in which ARG can be utilized in the retina is through arginase, which yields ORN and urea. High arginase has been associated with retinal neurodegeneration, neural dysfunction, and vascular injury in mice.(23) An impaired arginase pathway has also been found in humans with diabetic retinopathy who were shown to have higher levels of ORN and ARG than controls.(24,25) There was no difference in ORN concentrations between glaucoma patients and controls in the current study, however, there was a trend for increased ARG in glaucoma patients and in those with both glaucoma and tinnitus. This may suggest that the arginase pathway is of more interest than the NOS pathway in regard to ARG metabolism in glaucoma.

NO could also be involved in glaucoma through over-activity of NOS, which can lead to neurodegeneration and excitotoxicity.(26) As previously mentioned, over-excitation of the neurons by GLU could lead to a neurotoxic NO/ONOO⁻ loop, as shown in Figure 6.2.(27) Previous research has shown high levels of GLU in the vitreous body of humans and monkeys with glaucoma.(28) In rat and mouse models, GLU was also implicated in retinal ganglion cell death.(29,30) In the current study, GLU was slightly higher in glaucoma patients than in controls but this difference was not significant (median: 43.0 $\mu\text{mol/L}$ vs. 39.5 $\mu\text{mol/L}$, respectively).

Regarding tinnitus, research has shown that impairment in NO production may mediate ototoxicity in the early phase of tinnitus and subsequent NO-related oxidative damage could interfere in the inner ear in later phases.(31) GLU and GLN are the main excitatory neurotransmitters in the mammalian cochlea and high levels of these metabolites can lead to altered neural excitability.(15,32) Noise exposure can lead to excessive release of GLU between the inner hair cells and auditory nerve, leading to overexpression of NDMA and therefore too much calcium entering the nerve cell (see Figure 6.2), leading to nerve cell

death.(33) However, in the current study, patients with tinnitus did not show markers of excitotoxicity (Table 6.3).

A limitation of the research is that amino acids are sensitive to the way the samples are taken and stored. In one study, for blood samples left at room temperature for 6 hours, ORN and GLU concentrations increased approximately 50% compared to those analyzed immediately, while ARG decreased by about 50%. This effect was mitigated (although not completely) when samples were immediately placed in a 4°C environment.(34) Another study showed that ARG concentrations actually increased 2.5% per year while frozen.(35) However, in this study all samples were taken, immediately stored at -20°C, and analyzed within a few days, mitigating any of these sensitivities. A strong point of the study is the strict definition of glaucoma that was used to select the POAG patients. Another strong point of this study is the use of controls for comparison. In Figure 6.3 it can be seen that the results are not all distributed evenly within the reference ranges provided by the lab. Without controls for comparison, it could have erroneously been concluded that, for instance, glaucoma patients had relatively high GLN and ORN.

More research needs to be done to confirm if there is, in fact, NO production impairment in glaucoma and tinnitus. If so, this idea has potential future consequences for diagnosis and treatment. Blood tests could be included in the diagnosis of both pathologies, this could contribute an objective measurement to the subjective diagnosis of tinnitus. Regarding treatment, NO donating medication in the form of an eyedrop is already approved for the treatment of glaucoma for its IOP lowering effect, the vascular effect has to be further proven, but seems promising.(36) Possible treatments for oxidative stress and excitotoxicity in both diseases could come in the form of coenzyme Q10 (CoQ₁₀). CoQ10 inhibits free radical generation by maintaining the mitochondrial membrane potential. It has been found to promote retinal ganglion cell survival and improve tinnitus symptoms.(30,37) GLU receptor antagonists have also been used to mitigate glutamatergic excitation, potentially improving sensorineural tinnitus and showing promise for protection of visual loss in glaucoma.(16,38,39)

Diet may be another target point for treatment if NO bioavailability is, indeed, decreased in glaucoma and tinnitus. If the normal ARG pathway is impaired, NO can be produced through an alternative pathway through dietary intake of nitrates. In this pathway, nitrates found in foods such as kale and beetroot are reduced to nitrite by bacteria in the saliva. Nitrite is then further reduced to NO in the stomach due to the low pH level.(40) A high dietary nitrate intake has been shown to reduce the incidence of glaucoma by 20-30%.(41) A shortage of folic acid could also play a role in NO-related excitotoxicity because it is required to couple NOS with ARG, without it, free radicals are selectively produced.(42)

In conclusion, glaucoma patients and patients with both glaucoma and tinnitus seemed to have a slight increase in ARG but no other differences in concentrations of NO biomarkers. Future work should repeat this work in new, larger cohorts, perhaps with a particular focus on the arginase pathway of ARG metabolism. This could give a better understanding of the connection between both pathologies. Additionally, looking at genes involved in the production of NO in glaucoma patients with and without tinnitus may yield an underlying reason for why these changes occur in the first place.

ACKNOWLEDGEMENTS

We would like to thank W. Nieboer for his assistance with data collection and R. Heiner-Fokkema for the biomarker analysis and conversations about the results.

REFERENCES

1. Leske MC, Cristina Leske M. Ocular perfusion pressure and glaucoma: clinical trial and epidemiologic findings. *Current Opinion in Ophthalmology*. 2009. pp. 73–78. doi:10.1097/icu.0b013e32831eef82
2. Pasquale LR. Vascular and autonomic dysregulation in primary open-angle glaucoma. *Curr Opin Ophthalmol*. 2016;27: 94–101.
3. Galassi F, Renieri G, Sodi A, Ucci F, Vannozzi L, Masini E. Nitric oxide proxies and ocular perfusion pressure in primary open angle glaucoma. *Br J Ophthalmol*. 2004;88: 757–760.
4. Doganay S, Evereklioglu C, Turkoz Y, Er H. Decreased nitric oxide production in primary open-angle glaucoma. *Eur J Ophthalmol*. 2002;12: 44–48.
5. Huang W, Wang W, Zhou M, Zhang X. Association of single-nucleotide polymorphism rs4236601 near caveolin 1 and 2 with primary open-angle glaucoma: a meta-analysis. *Clin Experiment Ophthalmol*. 2014;42: 515–521.
6. Wiggs JL, Kang JH, Yaspan BL, Mirel DB, Laurie C, Crenshaw A, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma in Caucasians from the USA. *Hum Mol Genet*. 2011;20: 4707–4713.
7. Loiselle AR, Neustaeter A, de Kleine E, van Dijk P, Jansonius NM. Associations between tinnitus and glaucoma suggest a common mechanism: A clinical and population-based study. *Hear Res*. 2020;386: 107862.
8. Neri S, Signorelli S, Pulvirenti D, Mauceri B, Cilio D, Bordonaro F, et al. Oxidative stress, nitric oxide, endothelial dysfunction and tinnitus. *Free Radic Res*. 2006;40: 615–618.
9. Teranishi M, Uchida Y, Nishio N, Kato K, Otake H, Yoshida T, et al. Polymorphisms in genes involved in the free-radical process in patients with sudden sensorineural hearing loss and Ménière's disease. *Free Radic Res*. 2013;47: 498–506.
10. Zhao Y, Vanhoutte PM, Leung SWS. Vascular nitric oxide: Beyond eNOS. *J Pharmacol Sci*. 2015;129: 83–94.
11. Bryan NS, Loscalzo J. Nitrite and Nitrate in Human Health and Disease. *Humana Press*; 2017.
12. Underhill S, Amara S. TRANSPORTERS | Glutamate Transporters in Epilepsy. *Encyclopedia of Basic Epilepsy Research*. 2009. pp. 1398–1405. doi:10.1016/b978-012373961-2.00003-5
13. Atlante A, Calissano P, Bobba A, Giannattasio S, Marra E, Passarella S. Glutamate neurotoxicity, oxidative stress and mitochondria. *FEBS Lett*. 2001;497: 1–5.
14. Raul MTMA, Takumida M, Anniko M, Raul. Pharmacological Models for Inner Ear Therapy with Emphasis on Nitric Oxide. *Acta Oto-Laryngologica*. 2001. pp. 16–20. doi:10.1080/000164801300006218
15. Sahley TL, Nodar RH. A biochemical model of peripheral tinnitus. *Hear Res*. 2001;152: 43–54.
16. Imsuwansri T, Hoare DJ, Phaisaltuntiwongs W, Srisubat A, Snidvongs K. Glutamate receptor antagonists for tinnitus. *Cochrane Database of Systematic Reviews*. 2016. doi:10.1002/14651858.cd012391
17. Stanhewicz AE, Larry Kenney W. Role of folic acid in nitric oxide bioavailability and vascular endothelial function. *Nutrition Reviews*. 2017. pp. 61–70. doi:10.1093/nutrit/nuw053
18. Trotti D, Rossi D, Gjesdal O, Levy LM, Racagni G, Danbolt NC, et al.

-
- Peroxynitrite inhibits glutamate transporter subtypes. *J Biol Chem.* 1996;271: 5976–5979.
19. Heeg GP, Blanksma LJ, Hardus PLIJ, Jansonius NM. The Groningen Longitudinal Glaucoma Study. I. Baseline sensitivity and specificity of the frequency doubling perimeter and the GDx nerve fibre analyser. *Acta Ophthalmol Scand.* 2005;83: 46–52.
 20. van Vliet D, Bruinenberg VM, Mazzola PN, van Faassen MHJR, de Blaauw P, Kema IP, et al. Large Neutral Amino Acid Supplementation Exerts Its Effect through Three Synergistic Mechanisms: Proof of Principle in Phenylketonuria Mice. *PLoS One.* 2015;10: e0143833.
 21. Polak K, Luksch A, Berisha F, Fuchsjaeger-Mayrl G, Dallinger S, Schmetterer L. Altered nitric oxide system in patients with open-angle glaucoma. *Arch Ophthalmol.* 2007;125: 494–498.
 22. Kotikoski H, Moilanen E, Vapaatalo H, Aine E. Biochemical markers of the L-arginine-nitric oxide pathway in the aqueous humour in glaucoma patients. *Acta Ophthalmol Scand.* 2002;80: 191–195.
 23. Narayanan SP, Rojas M, Suwanpradit J, Toque HA, Caldwell RW, Caldwell RB. Arginase in retinopathy. *Prog Retin Eye Res.* 2013;36: 260–280.
 24. Sumarriva K, Uppal K, Ma C, Herren DJ, Wang Y, Chocron IM, et al. Arginine and Carnitine Metabolites Are Altered in Diabetic Retinopathy. *Invest Ophthalmol Vis Sci.* 2019;60: 3119–3126.
 25. Abhary S, Kasmeridis N, Burdon KP, Kuot A, Whiting MJ, Yew WP, et al. Diabetic retinopathy is associated with elevated serum asymmetric and symmetric dimethylarginines. *Diabetes Care.* 2009;32: 2084–2086.
 26. Toda N, Nakanishi-Toda M. Nitric oxide: ocular blood flow, glaucoma, and diabetic retinopathy. *Prog Retin Eye Res.* 2007;26: 205–238.
 27. Pall ML, Bedient SA. The NO/ONOO-cycle as the etiological mechanism of tinnitus. *Int Tinnitus J.* 2007;13: 99–104.
 28. Dreyer EB. Elevated Glutamate Levels in the Vitreous Body of Humans and Monkeys With Glaucoma. *Archives of Ophthalmology.* 1996. p. 299. doi:10.1001/archophth.1996.01100130295012
 29. Nucci C, Tartaglione R, Rombolà L, Morrone LA, Fazzi E, Bagetta G. Neurochemical evidence to implicate elevated glutamate in the mechanisms of high intraocular pressure (IOP)-induced retinal ganglion cell death in rat. *Neurotoxicology.* 2005;26: 935–941.
 30. Lee D, Shim MS, Kim K-Y, Noh YH, Kim H, Kim SY, et al. Coenzyme Q10 Inhibits Glutamate Excitotoxicity and Oxidative Stress-Mediated Mitochondrial Alteration in a Mouse Model of Glaucoma. *Investigative Ophthalmology & Visual Science.* 2014. p. 993. doi:10.1167/iovs.13-12564
 31. Raul MTMA, Takumida M, Anniko M, Raul. Pharmacological Models for Inner Ear Therapy with Emphasis on Nitric Oxide. *Acta Oto-Laryngologica.* 2001. pp. 16–20. doi:10.1080/000164801300006218
 32. Sedley W. Tinnitus: Does Gain Explain? *Neuroscience.* 2019. pp. 213–228. doi:10.1016/j.neuroscience.2019.01.027
 33. Figueiredo RR, Langguth B, Mello de Oliveira P, Aparecida de Azevedo A. Tinnitus treatment with memantine. *Otolaryngol Head Neck Surg.* 2008;138: 492–496.
 34. Schaefer A, Piquard F, Haberey P. Plasma amino-acids analysis: Effects of delayed samples preparation and of storage. *Clinica Chimica Acta.* 1987. pp. 163–169. doi:10.1016/0009-8981(87)90067-2
-

35. Hustad S, Eussen S, Midttun Ø, Ulvik A, van de Kant PM, Mørkrid L, et al. Kinetic Modeling of Storage Effects on Biomarkers Related to B Vitamin Status and One-Carbon Metabolism. *Clinical Chemistry*. 2012. pp. 402–410. doi:10.1373/clinchem.2011.174490
36. Garhöfer G, Schmetterer L. Nitric oxide: a drug target for glaucoma revisited. *Drug Discov Today*. 2019;24: 1614–1620.
37. Khan M, Gross J, Haupt H, Jainz A, Niklowitz P, Scherer H, et al. A pilot clinical trial of the effects of coenzyme Q10 on chronic tinnitus aurium. *Otolaryngology–Head and Neck Surgery*. 2007. pp. 72–77. doi:10.1016/j.otohns.2006.07.010
38. Sharma DK, Kaur S, Singh J, Kaur I. Role of acamprosate in sensorineural tinnitus. *Indian J Pharmacol*. 2012;44: 93–96.
39. Doozandeh A, Yazdani S. Neuroprotection in Glaucoma. *J Ophthalmic Vis Res*. 2016;11: 209–220.
40. Bryan NS, Loscalzo J. Nitrite and Nitrate in Human Health and Disease. Humana Press; 2017.
41. Kang JH, Willett WC, Rosner BA, Buys E, Wiggs JL, Pasquale LR. Association of Dietary Nitrate Intake With Primary Open-Angle Glaucoma: A Prospective Analysis From the Nurses' Health Study and Health Professionals Follow-up Study. *JAMA Ophthalmol*. 2016;134: 294–303.
42. Stanhewicz AE, Larry Kenney W. Role of folic acid in nitric oxide bioavailability and vascular endothelial function. *Nutrition Reviews*. 2017. pp. 61–70. doi:10.1093/nutrit/nuw053

Association of systemic medication exposure with glaucoma progression and glaucoma suspect conversion in the Groningen Longitudinal Glaucoma Study

Allison R. Loisel^{*}, Konstantinos Pappelis^{*}, Stijn Visser, Nomdo M. Jansonius

(Published: IOVS. November 2019; 60, 4548-4555)

ABSTRACT

The purpose of this study was to determine the association of statins, five classes of antihypertensive medication, and proton pump inhibitors with 1) primary open angle glaucoma (POAG) progression and 2) conversion of POAG suspects to POAG. We retrospectively investigated the records of a cohort with POAG cases and suspects from the Groningen Longitudinal Glaucoma Study. To quantify visual field (VF) deterioration in cases, we used the rate of progression of the mean deviation (MD). Suspects were considered to have converted at the time point after which two consecutive VF tests for at least one eye were abnormal (glaucoma hemifield test outside normal limits). Progression and conversion were analyzed with quantile and logistic regression, respectively, with the systemic medications as predictors, controlling for age, gender, BMI, pre-treatment intraocular pressure (IOP), corneal thickness, and baseline MD. The multivariable models were built with and without IOP intervention. No systemic medications were associated with POAG progression in the final IOP/treatment-adjusted or unadjusted model. However, angiotensin II receptor blockers (ARBs) appeared to slow progression in older patients ($b=0.014$, $P=0.0001$). ACE-inhibitors (ACEIs) were significantly associated with a decrease in POAG suspect conversion in both the IOP/treatment-adjusted and unadjusted model (OR=0.23, 95%CI: 0.07-0.79, $P=0.012$; OR=0.24, 95%CI: 0.07-0.78, $P=0.021$, respectively) as were ARBs (OR=0.12, 95%CI: 0.01-0.98, $P=0.014$; OR=0.11, 95%CI: 0.01-0.87, $P=0.005$, respectively). No overall association of VF progression with systemic medication was found; ARBs delayed progression in older patients. ACEIs and ARBs were associated with a lower risk of suspect conversion. The pathophysiology of this relationship is yet to be disentangled.

7.1 INTRODUCTION

Glaucoma is a chronic and progressive eye disease characterized by cupping of the optic disc, thinning of the retinal nerve fiber layer, loss of retinal ganglion cells (RGCs), and loss of visual function.^(1,2) A high intraocular pressure (IOP) is an important risk factor for glaucoma and IOP-lowering medications, laser, or surgery are currently the only available options to delay (but not inhibit) glaucomatous progression.^(3,4) However, not every person with ocular hypertension will convert to the disease, and glaucoma may also develop in those with an apparently normal IOP.⁽⁵⁾ These observations imply that other risk factors exist that are not being addressed in current treatment decisions.

Several other factors may contribute to the development and progression of the disease, including systemic medications used to treat other pathologies. Specifically, a protective role of statins, possibly unrelated to their cholesterol-lowering effect, has been reported in primary open-angle glaucoma (POAG).^(6–9) Moreover, numerous studies have examined the effect of antihypertensive (AH) medication on glaucoma, due to the fact that the disease is believed to contain a vascular component.^(10–12) This suggests that drugs able to modify vascular events could pertain to the outcome of glaucoma. In this regard, proton pump inhibitors (PPIs), a medication class used to lower the levels of stomach acid in gastric and esophageal pathologies, could influence unfavorably the course of the disease due to their documented inhibition of nitric oxide (NO) production.⁽¹³⁾ To make it even more complicated, these drugs could simultaneously have a beneficial effect because they create an unfavorable gastric environment for *Helicobacter pylori*, a bacterium for which an association with increased risk for POAG has been suggested.⁽¹⁴⁾

However, the results of studies examining the role of statins and AH medication in POAG are conflicting.^(15,16) A neuroprotective property of statins as well as of certain AH medications has been suggested, but not all studies confirm such an effect.^(17,18) Even from a pathophysiological perspective, although hypertension has been suggested as a risk factor for glaucoma, aggressive treatment with blood pressure (BP) medication could also result in ischemic damage to the RGCs mediated by a low diastolic blood pressure.⁽¹⁹⁾ In addition, to the best of our knowledge, there currently exists only scarce evidence addressing a potential effect of PPIs on POAG development. Lastly, some study designs in the literature incorporate a loose definition of POAG (e.g. prescription of glaucoma medication) based on national registries or insurance claim data.^(11,16) As a result, it is impossible to differentiate the impact of systemic medication on glaucoma suspects from the corresponding impact on those with a glaucoma diagnosis.

Therefore, the aim of this study was to determine the association of statins, five main classes of AH medication, and PPIs with 1) POAG progression and 2) conversion of POAG suspects to a POAG diagnosis. For this purpose, we retrospectively looked into the information of the Groningen Longitudinal Glaucoma Study (GLGS) to determine exposure to the aforementioned systemic medications in a cohort of POAG patients and POAG suspects.

7.2 METHODS

7.2.1 Study population

Patients were selected from the GLGS database. The GLGS began in 2000 and was originally a prospective, observational cohort study conducted in the clinical setting of the University Medical Center Groningen (UMCG), comprising both glaucoma patients and glaucoma suspects (predominantly Caucasians). In 2000, the UMCG served both as an academic and as a community hospital; the majority of patients in the current study should be considered community hospital patients. The objectives and methods of the GLGS have been previously described.(20,21) After the onset of the original GLGS, we continued adding newly diagnosed glaucoma patients, making the GLGS a dynamic population. From those patients who visited the clinic in 2015, we recorded their current and past systemic medications, and height and weight. This is the subset used in the current study. For the glaucoma patients, we associated the rate of progression (ROP; defined below) with systemic medication use, making the study design a retrospective follow-up study. For glaucoma suspects, we compared systemic medication use between those who converted to glaucoma and those who did not, making the study design a case-control study. The study protocol was approved by the ethics board of the University Medical Center Groningen and followed the tenets of the Declaration of Helsinki. All patients provided written informed consent.

In order to be eligible, subjects had to be followed with standard automated perimetry (see next subsection). Those with pseudoexfoliative or pigment dispersion glaucoma or a history of angle closure or secondary glaucoma were excluded (leaving POAG and POAG suspects). For being a glaucoma patient, glaucomatous visual field (VF) loss had to be present at baseline in at least one eye.(20) For glaucomatous baseline VF loss, two consecutive tests had to be abnormal (see next subsection) in at least one eye. Defects had to be compatible with glaucoma and without any other explanation. A VF test prior to the two baseline tests was discarded to reduce the influence of learning. Thus, at least three tests had to be performed at baseline before glaucomatous VF loss could be diagnosed. Glaucoma suspects were those who had an intact VF when entering the study and were followed in our outpatient department because of ocular hypertension (IOP above 20 mmHg on at least two separate visits), a positive family history of glaucoma (glaucoma reported in father, mother, brother, or sister), or a suspected optic disc (cup-to-disk ratio above 0.6), or combinations thereof.(22)

7.2.2 Perimetry, progression, and conversion

Perimetry was performed using the Humphrey Field Analyzer (HFA; Carl Zeiss, Jena, Germany) 30-2 Swedish interactive threshold algorithm (SITA) fast strategy with 30-2 grid. An abnormal test result was defined as a glaucoma hemifield test 'outside normal limits'. Test results were included only if they were reliable; a test result was considered unreliable if false positives exceeded 10% or if both false negatives and fixation losses exceeded 10% and 20%, respectively. We pooled false negatives and fixation losses because they were reported to have a much smaller influence on the mean deviation (MD) and the false negatives, especially, are not informative in glaucoma.(23,24)

In glaucoma patients, the rate of progression (ROP) was calculated as the slope of the MD over time, after a minimum of 5 years of perimetric follow-up.(25) For the conversion analysis, subjects who were glaucoma suspects were considered to have converted to a glaucoma diagnosis at the time point after which all subsequent VF tests (at least two) for at least one eye were abnormal.

7.2.3. Risk factors

The possible risk factors for glaucoma progression that were included in this study were: age at baseline, gender, body mass index (BMI), highest pre-treatment IOP, central corneal thickness (CCT), baseline MD (dichotomized above and below the median of -9.4 dB),(26) mean IOP during follow-up, surgery for glaucoma (Y/N), number of glaucoma medications, and systemic medications. Systemic medications included were statins, diuretics, angiotensin II receptor blockers (ARBs), ACE-inhibitors (ACEIs), calcium channel blockers (CCBs), beta blockers, and PPIs. For the number of glaucoma medications, topical and oral were included, and if the patient underwent laser treatment, this was considered as an additional medication.(27)

7.2.4. Systemic medication exposure ascertainment

To verify systemic medication exposure, the complete medical file of each patient was examined, in combination with a semi-structured interview that took place in 2015 (at or towards the end of the follow-up). An interviewer (blinded to the study question and taking no other part in the study procedures) asked each participant to list their current medications, guiding them with additional questions such as: “What medications are you currently taking for high blood pressure/high cholesterol/your heart/your stomach?”. To ascertain prior or subsequent use of the reported or other relevant medications we recorded all medication listings until the end of the patient’s follow-up from the patient’s hospital file, which included letters to their primary care doctors detailing prescribed medications. We defined the ‘oldest recorded exposure date’ as the very first date a drug appeared in this listing (or mentioned in the interview), while being absent from all listings of previous reports. We used this date 1) to approximate the duration of systemic medication use during follow-up for the progression analysis and 2) to ascertain that systemic medication exposure occurred prior to the conversion date in the conversion analysis.

7.2.5. Data analysis

For the progression analysis, only one eye per patient was included. If a patient met the inclusion criteria with both eyes, a randomly chosen eye was included. Conversion was based on a by-patient basis: those who converted with at least one eye were considered to have converted; for analysis the first converted eye was used and if no conversion took place, a random eye was chosen. The patient characteristics were described with mean and standard deviation (SD) for normally distributed variables. For variables with a skewed distribution, we used median and interquartile range (IQR) instead. In the multivariable analysis (see below), missing data for CCT (2 cases) and BMI (3 cases) were imputed from the median value. There were no other missing values for any other risk factor.

Due to the non-normality of the ROP distribution, quantile regression was performed using a saturated model with ROP as the outcome variable and all predictor variables included. The least significant variable was then removed and the models with and without the least significant variable were compared using the Akaike information criterion (AIC). If the model without the concerning variable was the better fit for the data, the same process was repeated for the next least significant variable, and this process was continued until we reached a minimal model. Coefficients and P-values were reported. The saturated model for the ROP regression can be found in the supplementary material (supplementary Table S1).

Conversion of glaucoma suspects to a glaucoma diagnosis was analyzed using a logistic regression model. The saturated model included all the predictor variables included above, except for glaucoma surgery and baseline MD, which would both be irrelevant for those not yet diagnosed with glaucoma. Mean IOP during follow-up was calculated either until the point at which the patient converted, or for the entire follow-up duration if the patient never converted. Again using the AIC, covariates were removed and a minimal model was created. Odds ratios (ORs) with 95% confidence intervals and P-values were reported. The saturated model for the conversion logistic regression model can be found in the supplementary material (supplementary Table S2).

All analyses were performed using R (version 3.3.3; R Foundation for Statistical Computing, Vienna, Austria). A P-value of 0.05 or less was considered statistically significant. According to the AIC, inclusion in the final model implies $P < 0.16$.

7.3 RESULTS

We included 250 patients in the ROP analysis and 112 glaucoma suspects for the conversion analysis. Of the 112 glaucoma suspects, 21 were included because of ocular hypertension, 4 because of a positive family history of glaucoma, 6 because of a suspected optic disc, and 81 because of combinations thereof. Table 7.1 shows the characteristics of the study population.

Median follow-up duration of the glaucoma patients was 12 years; median ROP -0.27 dB/year. Table 7.2 depicts the univariable quantile regression analysis with median ROP as the dependent variable. Older age, lower BMI, lower IOP before treatment, and a higher number of glaucoma medications were significantly associated with faster progression.

Table 7.1 | Demographics of the study population

	Glaucoma patients	Glaucoma Suspects	
		Converted	Not converted
Number of patients	250	53	59
Age at baseline (years; mean $\pm$ SD)	61.8 $\pm$ 9.9	58.3 $\pm$ 10.0	55.4 $\pm$ 11.3
Gender (% Female)	46.0%	56.6%	54.2%
BMI (kg/m ² ; mean $\pm$ SD)	26.2 $\pm$ 4.2	25.6 $\pm$ 3.8	27.2 $\pm$ 4.5
IOP before treatment (mmHg; median [IQR])	26.0 (22.0 to 31.0)	26.0 (23.0,30.0)	29.0 (24.0,32.0)
CCT (μ m; median [IQR])	537.0 (512.5 to 566.0)	543.0 (519.0 to 558.0)	551.0 (539.5 to 576.0)
VF MD at baseline (dB; median [IQR])	-9.4 (-15.6 to -5.1)	-1.1 (-3.9 to 0.0)	-0.8 (-1.8 to 0.2)
Follow-up duration (yrs; median [IQR])	12.0 (9.0 to 15.0)	7.0 (4.0 to 12.0)	16.0 (13.0 to 18.0)
Mean IOP during follow-up (mmHg; median [IQR])	13.2 (11.3 to 15.5)	15.5 (13.9 to 17.3)	17.1 (14.6 to 18.2)
ROP (dB/year; median [IQR])	-0.27 (-0.55 to -0.08)	NA	NA
Statins (% [n])	37.2% (93)	15.1% (8)	40.7% (24)
Statin duration (percentage of follow-up years; median [IQR])	45.6% (25.3 to 82.5)	NA	NA
Diuretics (% [n])	27.6% (69)	9.4% (5)	33.9% (20)
Angiotensin II receptor blockers (% [n])	15.2% (38)	1.9% (1)	15.2% (9)
ACE-inhibitors (% [n])	24.8% (62)	7.5% (4)	25.4% (15)
Calcium channel blockers (% [n])	19.2% (48)	11.3% (6)	20.3% (12)
Beta blockers (% [n])	27.2% (68)	18.8% (10)	30.5% (18)
AH duration (percentage of follow-up years; median [IQR])	50.0% (27.3 to 100.0)	NA	NA
Proton pump inhibitors (% [n])	33.2% (83)	22.6% (12)	32.2% (19)
PPI duration (percentage of follow-up years; median [IQR])	44.4% (25.8 to 90.4)	NA	NA

SD standard deviation, *BMI* body mass index, *IOP* intraocular pressure, *CCT* central corneal thickness, *VF MD* standard automated perimetry mean deviation, *AH* antihypertensive, *NA* not applicable.

Table 7.2 | Univariable quantile regression analysis for glaucoma patients with median ROP as dependent variable

	Coefficient	P-value
Age (yrs)	-0.006	0.003
Gender (female)	0.080	0.15
BMI (kg/m ²)	0.010	0.050
IOP before treatment (mmHg)	0.005	0.008
CCT (μm)	-0.0002	0.76
VF MD at baseline (dB)	0.000	1.0
Mean IOP during follow-up (mmHg)	-0.005	0.52
Glaucoma surgery (0=No)	0.040	0.44
Number of glaucoma medications	-0.046	0.028
Statins	-0.060	0.27
Diuretics	-0.010	0.87
Angiotensin II receptor blockers	0.020	0.74
ACE inhibitors	-0.060	0.23
Calcium channel blockers	-0.080	0.31
Beta blockers	-0.080	0.16
Proton pump inhibitors	0.010	0.86

BMI body mass index, *IOP* intraocular pressure, *CCT* central corneal thickness, *VF MD* standard automated perimetry mean deviation.

Table 7.3 shows the final multivariable model for median ROP, along with a model built without the variables that account for intervention by the treating ophthalmologist (by excluding mean IOP during follow-up, number of glaucoma medications, and glaucoma surgery from the saturated model) to account for any systemic medication-mediated IOP changes. In both final models, older age and male gender were significantly associated with faster progression. Specifically, for each 10 years of age, the median ROP was 0.07 dB/year faster (0.06 dB/year for the model excluding intervention). Statins and ARBs remained in the model but were not significantly associated with ROP.

For these two systemic medications, we completed a secondary dose-response relationship analysis in which we created categories based on duration of medication use, defined as: no use, duration of use below the median, and duration of use above the median. Compared to non-users of statins, there was a trend toward faster progression with prolonged statin use (6 years or less, $b=-0.083$, $P=0.06$; more than 6 years, $b=-0.130$, $P=0.009$). There was no dose-response relationship found for ARBs.

We also repeated the analysis using a variable with the cumulative number of AHs (in place of the different classes) as a marker for the severity of hypertension, but this variable was the first to leave the model, suggesting the number of AHs was not associated with progression.

Interactions between the two systemic medications remaining in the final model (ARBs and statins) and both pretreatment IOP and age were also investigated, with a Bonferroni-

adjusted significance cutoff of $P=0.0125$. The only significant interaction effect on ROP was between ARBs and age ($b=0.014$, $P=0.0001$). For each additional 10 years of age, ARB takers had a median ROP that was 0.14 dB/year slower than non ARB takers. Additionally, tests for collinearity between systemic medications revealed only a moderate collinear relationship between beta blockers and ACEIs ($\phi = 0.42$, $P<0.001$), all other ϕ coefficients were similar or lower.

Table 7.3 | Final models for glaucoma patients with median ROP as dependent variable

	Final Model		Final Model (not adjusted for IOP intervention)	
	Coefficient	P-value	Coefficient	P-value
Age (yrs)	-0.007	0.001	-0.006	0.010
Gender (female)	0.122	0.018	0.128	0.004
BMI (kg/m ²)	0.010	0.032	0.006	0.14
IOP before treatment (mmHg)	0.004	0.12	0.004	0.077
CCT (μ m)	-0.0008	0.14	-0.0007	0.16
Number of glaucoma medications	-0.036	0.056	NA	NA
Statins	-0.083	0.086	NA	NA
Angiotensin II receptor blockers	0.089	0.097	NA	NA

BMI body mass index, *IOP* intraocular pressure, *CCT* central corneal thickness.

Of the 112 glaucoma suspects, 53 converted during follow-up. The median follow-up duration until conversion was 7 years for those who converted; those who did not convert were followed for a median of 16 years. The survival curve is displayed in Figure 7.1. From this curve, it can be predicted that it will take 15 years for 50% of the suspects to convert. Tables 7.4 and 7.5 present the univariable and final multivariable logistic regression models, respectively, for glaucoma suspect conversion. In the univariable analysis, a higher BMI, a higher number of glaucoma medications, and the use of statins, diuretics, ARBs, and ACEI were associated with a significant decrease in conversion. Again, multivariable models were built with and without mean IOP during follow-up and number of glaucoma medications. In the final model, a higher number of glaucoma medications and the use of ARBs and ACEI were associated with a significant decrease in conversion to a glaucoma diagnosis. Even in the final model without mean IOP and IOP treatment, the rate of conversion of suspects on ARBs and ACEIs was 89% and 76% lower, respectively, than those not taking the drugs, suggesting the possible protective association is not mediated by IOP. Tests for collinearity between systemic medications revealed a moderate collinear relationship between beta blockers and CCBs ($\phi = 0.53$, $P<0.001$), all other ϕ coefficients were similar or lower.

FIG 7.1 Survival curve for the glaucoma suspect population. Conversion to POAG is defined as the time point after which all subsequent VF tests (at least two) for at least one eye were abnormal.

Table 7.4 | Univariable analysis with glaucoma suspect conversion as dependent variable

	OR (95% CI)	P-value
Age (yrs)	1.03 (0.99,1.06)	0.14
Gender (female)	1.1 (0.52,2.32)	0.80
BMI (kg/m ²)	0.91 (0.83,1.00)	0.043
IOP before treatment (mmHg)	0.98 (0.94,1.03)	0.44
CCT (μm)	0.99 (0.98,1.00)	0.058
Mean IOP during follow-up (mmHg)	0.90 (0.80,1.01)	0.078
Number of glaucoma medications	0.53 (0.34,0.85)	0.004
Statins	0.26 (0.1,0.65)	0.002
Diuretics	0.20 (0.07,0.59)	0.001
Angiotensin II receptor blockers	0.11 (0.01,0.87)	0.008
ACE-inhibitors	0.24 (0.07,0.78)	0.009
Calcium channel blockers	0.50 (0.17,1.44)	0.19
Beta blockers	0.53 (0.22,1.28)	0.15
Proton pump inhibitors	0.62 (0.26,1.43)	0.26

OR Odds ratio, CI confidence interval, BMI body mass index, IOP intraocular pressure, CCT central corneal thickness.

Table 7.5 | Final models with glaucoma suspect conversion as dependent variable

	Final Model		Final Model (not adjusted for IOP intervention)	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (yrs)	NA	NA	1.03 (0.99,1.07)	0.14
BMI (kg/m ²)	NA	NA	0.93 (0.83,1.07)	0.15
CCT (μ m)	0.99 (0.98,1.00)	0.063	0.99 (0.98,1.00)	0.069
Number of glaucoma medications	0.59 (0.37,0.94)	0.025	NA	NA
Angiotensin II receptor blockers	0.12 (0.01, 0.98)	0.014	0.11 (0.01,0.87)	0.005
ACE inhibitors	0.23 (0.07,0.79)	0.012	0.24 (0.07,0.78)	0.021

OR Odds ratio, CI confidence interval, BMI body mass index, CCT central corneal thickness.

7.4 DISCUSSION

No systemic medications were associated with POAG progression in the final IOP-intervention adjusted or unadjusted model; statins and ARBs remained in the final IOP-intervention adjusted model without reaching significance, but an interaction term revealed a significantly slower progression associated with ARBs for older ages. ACEIs and ARBs were significantly associated with a decrease in POAG suspect conversion in the final model, regardless of IOP-intervention adjustment.

Regarding the effect of statins on POAG progression, other studies have given varying results. Iskadjian et al. reported no significant differences in the need for adjunct topical IOP-lowering medications between statin users and non-users.(12) Leung et al. found that significantly more non-progressors were using statins. In contrast to our study, their study concerned only normal tension glaucoma (NTG) subjects (256 subjects followed for 3 years; 31 taking statins).(8) An interaction term between statin use and pretreatment IOP added to our model did not uncover a significant association, suggesting that statins don't have a selective association with NTG in our study population. De Castro et al. assessed progression with structural parameters and reported that statin users — albeit only the ones not receiving aspirin treatment simultaneously — had lower progression rates (n=76; smallest group n=12; follow-up duration: 5.5 years).(28) This observation suggests that early structural changes might be more sensitive to the effect of statins compared to VF parameters, but a study assessing both structure and function would be needed to clarify this hypothesis. McCann et al. could not perform a meta-analysis on these studies because the definitions of glaucoma differed.(15) Lastly, a propensity score analysis by Whigham et al. reported

that a history of statin use resulted in slower VF progression (847 subjects followed for 3.5 years; 629 taking statins).(6) However, their propensity score was limited to age, gender, baseline glaucomatous severity, and systemic medical conditions; hence, it did not include some important variables present in our analysis, especially the variables related to IOP. Notably, in our study, the relevant ROP coefficient for the IOP-intervention adjusted model suggests a faster progression (-0.083 dB/year for statin users compared to non-users), but this result did not reach significance ($p=0.086$). Our secondary dose-response analysis suggested faster progression for POAG patients using statins for more than 6 years (-0.130 dB/year compared to non-users, $p=0.009$), but according to a recent publication this is more likely a result of higher serum cholesterol.(29) All these studies, including ours, were observational studies, that is, the statins were not prescribed as part of a randomized control trial (RCT).

With regards to statin use and POAG incidence, most observational studies, but not all, agree that short-term statin use (2 years or less) does not significantly affect the risk.(7,16,30–33) Among them, Stein et al. also examined the conversion of glaucoma suspects ($n=49,628$) within a retrospective longitudinal cohort design. They found that POAG suspects who used statins for 1 or 2 years had a smaller hazard for conversion ($HR=0.907$, 95% CI: $0.846-0.973$), but not for the need for glaucoma surgery.(7) It is possible, of course, that any beneficial effect, is only result of longer exposure to the medication.(7,30,32) Interestingly, a large observational prospective study by Talwar et al. (25420 subjects; 15898 taking statins) showed reduced POAG incident risk after 2 years of statin use ($HR=0.79$, 95% CI: $0.66-0.96$), independent of the cholesterol-lowering effect, dosage, and statin type.(9) It has been suggested that this effect may be mediated by neuroprotective mechanisms.(17,34,35) Albeit significant in these studies with very large sample sizes, the effect sizes were actually small. This illustrates that a visible effect size in large samples does not immediately imply clinical relevance. Notably, in the univariate analysis of our study, statins are shown to be associated (after Bonferroni correction) with a reduced odds ratio for suspect conversion ($OR=0.26$, 95% CI: $0.1-0.65$, $p[\text{adjusted}]=0.014$); however, the multivariate model reveals that this association is mediated by concomitant use of AH medications.

A recent observational study from insurance claim data suggested a causal, protective effect of AH medication, especially those in the renin-angiotensin category, with respect to incident glaucoma.(11) Hirooka et al. reported a similar finding in a NTG sample treated with ACEIs, while Yang et al. found a neuroprotective effect of ARBs in a rat model.(18,36) The authors hypothesized that the decrease in angiotensin II levels reduces the activity of the NADPH-dependent oxidase complex, thus reducing oxidative stress and the subsequent RGC apoptosis. They also discussed that the increase in plasma bradykinin caused by the ACEIs offers extra possibilities: improved blood flow through activation of the L-arginine NO pathway, or protection from glutamate-induced neurotoxicity. In our study, ACEIs and ARBs were significantly associated with lower odds of suspects converting to POAG, which is in agreement with these results. In addition, the highly significant interaction term between age and ARBs in our progression analysis suggests that the older the glaucomatous patient, the more they could benefit from an ARB, as far as their glaucomatous progression is concerned.

The effects of other AH medications are less clear. Beta blockers have been associated with decreased POAG incidence in some studies, whereas the opposite has been reported for CCBs and, sometimes, even ARBs.(16,37,38) Diuretics were associated with increased conversion risk of ocular hypertensives in the European Glaucoma Prevention Study and increased risk of POAG in other studies.(31,38,39) A protective effect of the cumulative number of AH drugs was reported by Horwitz et al; additionally, Iskudjian et al. showed that the use of AH medication reduced the need for adjunct topical IOP-lowering medication.(11,12) Overall, these results suggest that AH medication classes might contribute differently to the course of glaucoma; this points towards the fact that pathways other than the BP-lowering effect could be involved.(40,41) Protection could be mediated by an IOP-lowering effect;(42–46) additional neuroprotective mechanisms have been attributed to medication in the renin-angiotensin category, as discussed in the previous paragraph.(47–49)

There are several reasons for these conflicting results. POAG treatment is a dynamic procedure in which the clinician's decisions interfere with the true (untreated) disease progression. Therefore, explanatory variables such as mean IOP during follow-up or number of glaucoma medications could mediate or antagonize effects observed in other studies. Furthermore, a threshold effect could exist for certain AH drugs, or a ceiling effect with no further increase in risk for glaucomatous damage after this ceiling has been reached, hampering consistent dose-response findings.(16,37) Lastly, it is possible that AH treatment is beneficial only when started timely and harmful if started after many years of untreated hypertension. This, however, is impossible to address in an observational study with limited information regarding the time course of blood pressure and its treatment. Interestingly, overtreatment of hypertension leading to hypotension has been reported as a risk factor for glaucoma.(19)⁽⁵⁰⁾ Noticeably, data extracted from a large cohort study (LifeLines Biobank) reveal that aggressive AH treatment is hardly the case in our region, the northern Netherlands.(51) Indeed, average (SD) SBP/DBP values for users and non-users of AH medication are, within Lifelines, 135(17)/79(10) and 128(15)/76(9), respectively.

PPIs appear to both mitigate and worsen glaucoma. In a study investigating systemic medication use and glaucoma in insurance claims data,(16) it was found that esomeprazol (a PPI), reduced the risk of POAG. This finding was of borderline significance but the authors hypothesized that it could be due to a reduction in *Helicobacter pylori* rates, and therefore reduced risk of POAG.(14,52) However, PPIs can also decrease production of NO, a vasodilator that plays a role in IOP regulation. POAG patients may already have a genetic and dietary susceptibility to reduced NO bioavailability.(53) In this regard, PPIs may be beneficial to those who do not have alterations in genes responsible for NO production, and harmful to those who do.

This study is limited by its retrospective design and its relatively small sample size. For medication ascertainment our primary metric was “any use during follow-up” (a commonly used metric in pharmacoepidemiology), but we added duration of use information for the progression analysis and temporal information for the conversion analysis (by ascertaining that systemic medication exposure occurred prior to the conversion date). Obviously, this metric is still limited by the absence of dosage information. Furthermore, we did not have BP

measurements and for this reason we could not perform a mediation analysis for BP. Instead, we used the number of BP medications as a crude proxy of disease severity. In spite of that, it is true that monotherapy is the traditional initial therapy for hypertension and subsequent medications are added depending on BP target and disease severity. The absence of BP measurements also did not allow us to examine overtreatment of hypertension, a situation that could lead to underperfusion of the RGCs and, hence, mask any protective effects. Nevertheless, our positive findings remain somewhat robust to this limitation, as they all lie on the protective side, whereas overtreatment is expected to have a negative effect on glaucoma progression or conversion. In addition, our population was predominantly Caucasian, so our results cannot be safely generalized, especially due to differential responses to cardiovascular agents among patients of different genetic ancestries. Lastly, our population's median age leans towards the younger side with regards to glaucoma populations, a limitation which we tried to address by assessing the interactions between systemic medication exposure and age in the models.

The main strength of this study is the long follow-up duration of the population. Furthermore, POAG patients and suspects were defined according to strict criteria, rather than being based on data from national registry or insurance claims. This ensures that the two groups are not cross-contaminated and that no other type of glaucoma is present in the dataset. Moreover, a novelty of this study is controlling the analysis for glaucoma medications, the surgical profile of each participant (operated/not operated), and mean IOP during follow-up. We believe that this reflects reality better, as it simulates the dynamic context of clinicians intervening in the process by trying to slow the glaucomatous progression.

Our study suggests that, within a glaucoma or glaucoma suspect population with regular follow-ups and well-controlled IOP, there exists some extra benefit added by BP medication, but its clinical relevance is unclear. A better study design and a larger sample size are both needed to strengthen these findings. Also interesting from a clinical perspective, a high pretreatment IOP is paradoxically almost beneficial on ROP (stays in the final model, but does not reach significance), whereas most studies show the opposite effect or no effect whatsoever.(26,54,55) This finding indicates that, in this population, the alarm of a higher baseline IOP could have resulted in early diagnosis and efficient, possibly more aggressive, treatment. As such, it is a marker of clinician's responsiveness that is visible due to the observational study design rather than a disease property.

In this study, approximately 47% of glaucoma suspects converted to glaucoma within a median follow-up of 16 years. According to the Ocular Hypertension Treatment Study (OHTS), the expected percentage of converted cases within 5 years is 5-10%, so — assuming a linear relation — less than 30% of our suspects should have converted.(56) A possible explanation for this discrepancy could be the fact that a healthy optic disc at baseline was additionally required in the OHTS - we also included suspects based on a suspected optic disc (47 of 112). Additionally, a positive family history was present in 44% of the participants in OHTS, to be compared with 58% in our study.(57) Together, these differences suggest that our participants were already “further down the line” at baseline. Also, the OHTS used a stricter criterion for conversion (three consecutive abnormal last VF tests, rather

than two); however, all of the converted suspects in our study actually had three consecutive abnormal last VF tests, as well. It must also be noted that 6 out of 59 non-converted suspects in our study had (only) one abnormal last VF, but this was not enough to classify them as ‘converted’, since most of these cases are expected to yield a healthy VF on their next visit. (58) Our converters fulfilled the functional criteria of the OHTS, but we did not have data to also include the structural criteria of the OHTS. As such, the difference in conversion rate between both studies is larger than reported above.

In conclusion, we found no overall significant association of glaucomatous VF progression with systemic medication exposure, but ARBs appeared to significantly delay progression in older patients. ACEIs and ARBs were significantly associated with a lower risk of suspect conversion to POAG. Since this study was limited by its design, further investigations, ideally RCTs, are needed to examine these relationships; should they be proven true, their exact pathophysiology is yet to be disentangled.

Table S1 | Saturated model with median ROP as dependent variable

	Coefficient	P-value
Age (yrs)	-0.007	<0.001
Gender (female)	0.115	0.014
BMI (kg/m ²)	0.005	0.29
IOP before treatment (mmHg)	0.005	0.062
CCT (μm)	-0.0007	0.12
VF MD at baseline (0 = below median MD)	-0.016	0.71
Mean IOP during follow-up (mmHg)	-0.005	0.49
Glaucoma Surgery (0=No)	-0.020	0.69
Number of glaucoma medications	-0.037	0.041
Statins	-0.031	0.54
Diuretics	-0.009	0.88
Angiotensin II receptor blockers	0.102	0.063
ACE-inhibitors	-0.028	0.54
Calcium channel blockers	-0.085	0.25
Beta blockers	0.046	0.41
Proton pump inhibitors	-0.052	0.32

BMI body mass index, *IOP* intraocular pressure, *CCT* central corneal thickness, *VF MD* standard automated perimetry mean deviation.

Table S2 | Saturated model with glaucoma suspect conversion as dependent variable

	OR (95% CI)	P-value
Age (yrs)	1.02 (0.98,1.07)	0.29
Gender (female)	1.12 (0.45,2.81)	0.80
BMI (kg/m ²)	0.97 (0.86,1.10)	0.60
IOP before treatment (mmHg)	1.01 (0.95,1.08)	0.65
CCT (μm)	0.99 (0.98,1.00)	0.11
Mean IOP during follow-up (mmHg)	0.94 (0.81,1.09)	0.42
Number of glaucoma medications	0.66 (0.40,1.09)	0.093
Statins	0.54 (0.14,2.12)	0.38
Diuretics	0.44 (0.10,1.96)	0.27
Angiotensin II receptor blockers	0.15 (0.01,1.75)	0.09
ACE inhibitors	0.37 (0.07,1.78)	0.20
Calcium channel blockers	1.70 (0.32,9.19)	0.54
Beta blockers	1.23 (0.28,5.44)	0.78
Proton pump inhibitors	1.08 (0.37,3.14)	0.89

OR Odds ratio, *CI* confidence interval, *BMI* body mass index, *IOP* intraocular pressure, *CCT* central corneal thickness.

REFERENCES

1. Airaksinen PJ, Alanko HI. Effect of retinal nerve fibre loss on the optic nerve head configuration in early glaucoma. *Graefes Arch Clin Exp Ophthalmol*. 1983;220(4):193–6.
2. Schuman JS. Quantification of Nerve Fiber Layer Thickness in Normal and Glaucomatous Eyes Using Optical Coherence Tomography. *Arch Ophthalmol*. 1995;113(5):586.
3. Garway-Heath DF, Crabb DP, Bunce C, Lascaratos G, Amalfitano F, Anand N, et al. Latanoprost for open-angle glaucoma (UKGTS): a randomised, multicentre, placebo-controlled trial. *Lancet*. 2015 Apr 4;385(9975):1295–304.
4. Heijl A, Leske MC, Bengtsson B, Hyman L, Bengtsson B, Hussein M, et al. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Arch Ophthalmol*. 2002 Oct;120(10):1268–79.
5. Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham EJ, Johnson CA, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002 Jun;120(6):714–20; discussion 829–30.
6. Whigham B, Oddone EZ, Woolson S, Coffman C, Rand Allingham R, Shieh C, et al. The influence of oral statin medications on progression of glaucomatous visual field loss: A propensity score analysis. *Ophthalmic Epidemiol*. 2017;25(3):207–14.
7. Stein JD, Newman-Casey PA, Talwar N, Nan B, Richards JE, Musch DC. The relationship between statin use and open-angle glaucoma. *Ophthalmology*. 2012 Oct;119(10):2074–81.
8. Leung DY, Li FCH, Kwong YYY, Tham CCY, Chi SCC, Lam DSC. Simvastatin and disease stabilization in normal tension glaucoma: a cohort study. *Ophthalmology*. 2010 Mar;117(3):471–6.
9. Talwar N, Musch DC, Stein JD. Association of Daily Dosage and Type of Statin Agent With Risk of Open-Angle Glaucoma. *JAMA Ophthalmol*. 2017 Mar 1;135(3):263–7.
10. Topouzis F. The role of ocular perfusion pressure in glaucoma. *Acta Ophthalmol*. 2014;92:0–0.
11. Horwitz A, Klemp M, Jeppesen J, Tsai JC, Torp-Pedersen C, Kolko M. Antihypertensive Medication Postpones the Onset of Glaucoma: Evidence From a Nationwide Study. *Hypertension*. 2017 Feb;69(2):202–10.
12. Iskedjian M, Walker JH, Desjardins O, Robin AL, Covert DW, Bergamini MVW, et al. Effect of selected antihypertensives, antidiabetics, statins and diuretics on adjunctive medical treatment of glaucoma: a population based study. *Curr Med Res Opin*. 2009 Aug;25(8):1879–88.
13. Lundberg JO, Weitzberg E, Lundberg JM, Alving K. Intragastric nitric oxide production in humans: measurements in expelled air. *Gut*. 1994 Nov;35(11):1543–6.
14. Zeng J, Liu H, Liu X, Ding C. The Relationship Between Helicobacter pylori Infection and Open-Angle Glaucoma: A Meta-Analysis. *Invest Ophthalmol Vis Sci*. 2015 Aug;56(9):5238–45.
15. McCann P, Hogg RE, Fallis R, Azuara-Blanco A. The Effect of Statins on Intraocular Pressure and on the Incidence and Progression of Glaucoma: A Systematic Review and Meta-Analysis. *Invest Ophthalmol Vis Sci*. 2016 May 1;57(6):2729–48.

16. Zheng W, Dryja TP, Wei Z, Song D, Tian H, Kahler KH, et al. Systemic Medication Associations with Presumed Advanced or Uncontrolled Primary Open-Angle Glaucoma. *Ophthalmology*. 2018 Jul;125(7):984–93.
17. Nagaoka T, Takahashi A, Sato E, Izumi N, Hein TW, Kuo L, et al. Effect of systemic administration of simvastatin on retinal circulation. *Arch Ophthalmol*. 2006 May;124(5):665–70.
18. Yang H, Hirooka K, Fukuda K, Shiraga F. Neuroprotective Effects of Angiotensin II Type 1 Receptor Blocker in a Rat Model of Chronic Glaucoma. *Investigative Ophthalmology & Visual Science*. 2009;50(12):5800.
19. Topouzis F, Roy Wilson M, Harris A, Founti P, Yu F, Anastasopoulos E, et al. Association of Open-angle Glaucoma With Perfusion Pressure Status in the Thessaloniki Eye Study. *Am J Ophthalmol*. 2013;155(5):843–51.e1.
20. Heeg GP, Blanksma LJ, Hardus PLLJ, Jansonius NM. The Groningen Longitudinal Glaucoma Study. I. Baseline sensitivity and specificity of the frequency doubling perimeter and the GDx nerve fibre analyser. *Acta Ophthalmol Scand*. 2005 Feb;83(1):46–52.
21. Wesselink C, Heeg GP, Jansonius NM. Glaucoma monitoring in a clinical setting: glaucoma progression analysis vs nonparametric progression analysis in the Groningen Longitudinal Glaucoma Study. *Arch Ophthalmol*. 2009 Mar;127(3):270–4.
22. Heeg GP, Jansonius NM. The groningen longitudinal glaucoma study III. The predictive value of frequency-doubling perimetry and GDx nerve fibre analyser test results for the development of glaucomatous visual field loss. *Eye*. 2009 Aug;23(8):1647–52.
23. Junoy Montolio FG, Wesselink C, Gordijn M, Jansonius NM. Factors that influence standard automated perimetry test results in glaucoma: test reliability, technician experience, time of day, and season. *Invest Ophthalmol Vis Sci*. 2012 Oct 9;53(11):7010–7.
24. Bengtsson B, Heijl A. False-negative responses in glaucoma perimetry: indicators of patient performance or test reliability? *Am J Ophthalmol*. 2000 Nov;130(5):689.
25. Jansonius NM. On the accuracy of measuring rates of visual field change in glaucoma [Internet]. Vol. 94, *British Journal of Ophthalmology*. 2010. p. 1404–5. Available from: <http://dx.doi.org/10.1136/bjo.2009.164897>
26. Leske MC, Heijl A, Hyman L, Bengtsson B, Dong L, Yang Z, et al. Predictors of long-term progression in the early manifest glaucoma trial. *Ophthalmology*. 2007 Nov;114(11):1965–72.
27. Garg A, Vickerstaff V, Nathwani N, Garway-Heath D, Konstantakopoulou E, Ambler G, et al. Primary Selective Laser Trabeculoplasty for Open Angle Glaucoma and Ocular Hypertension: Clinical Outcomes, Predictors of Success and Safety from the Laser in Glaucoma and Ocular Hypertension (LiGHT) Trial. *Ophthalmology* [Internet]. 2019 Apr 24; Available from: <http://dx.doi.org/10.1016/j.optha.2019.04.012>
28. De Castro DK, Punjabi OS, Bostrom AG, Stamper RL, Lietman TM, Ray K, et al. Effect of statin drugs and aspirin on progression in open-angle glaucoma suspects using confocal scanning laser ophthalmoscopy. *Clin Experiment Ophthalmol*. 2007 Aug;35(6):506–13.
29. Kang JH, Boumenna T, Stein JD, Khawaja

-
- A, Rosner BA, Wiggs JL, et al. Association of Statin Use and High Serum Cholesterol Levels With Risk of Primary Open-Angle Glaucoma. *JAMA Ophthalmol.* 2019 Jul 1;137(7):756–65.
30. McGwin G Jr, McNeal S, Owsley C, Girkin C, Epstein D, Lee PP. Statins and other cholesterol-lowering medications and the presence of glaucoma. *Arch Ophthalmol.* 2004 Jun;122(6):822–6.
 31. Owen CG, Carey IM, Shah S, de Wilde S, Wormald R, Whincup PH, et al. Hypotensive Medication, Statins, and the Risk of Glaucoma. *Investigative Ophthalmology & Visual Science.* 2010;51(7):3524.
 32. Marcus MW, Rogier P H, Ramdas WD, Wolfs RCW, De Jong PTVM, Vingerling JR, et al. Cholesterol-Lowering Drugs and Incident Open-Angle Glaucoma: A Population-Based Cohort Study. *PLoS One.* 2012;7(1):e29724.
 33. Chen H-Y, Hsu S-Y, Chang Y-C, Lin C-C, Sung F-C, Chen W-C, et al. Association Between Statin Use and Open-angle Glaucoma in Hyperlipidemia Patients: A Taiwanese Population-based Case-control Study. *Medicine .* 2015 Nov;94(45):e2018.
 34. Morishita S, Oku H, Horie T, Tonari M, Kida T, Okubo A, et al. Systemic simvastatin rescues retinal ganglion cells from optic nerve injury possibly through suppression of astroglial NF- κ B activation. *PLoS One.* 2014 Jan 2;9(1):e84387.
 35. Krempler K, Schmeer CW, Isenmann S, Witte OW, Löwel S. Simvastatin improves retinal ganglion cell survival and spatial vision after acute retinal ischemia/reperfusion in mice. *Invest Ophthalmol Vis Sci.* 2011 Apr 20;52(5):2606–18.
 36. Hirooka K, Baba T, Fujimura T, Shiraga F. Prevention of visual field defect progression with angiotensin-converting enzyme inhibitor in eyes with normal-tension glaucoma. *Am J Ophthalmol.* 2006 Sep;142(3):523–5.
 37. Müskens RPHM, de Voogd S, Wolfs RCW, Witteman JCM, Hofman A, de Jong PTVM, et al. Systemic antihypertensive medication and incident open-angle glaucoma. *Ophthalmology.* 2007 Dec;114(12):2221–6.
 38. Langman MJS, Lancashire RJ, Cheng KK, Stewart PM. Systemic hypertension and glaucoma: mechanisms in common and co-occurrence. *Br J Ophthalmol.* 2005 Aug;89(8):960–3.
 39. Miglior S, Torri V, Zeyen T, Pfeiffer N, Vaz JC, Adamsons I, et al. Intercurrent factors associated with the development of open-angle glaucoma in the European glaucoma prevention study. *Am J Ophthalmol.* 2007 Aug;144(2):266–75.
 40. Khawaja AP. Calcium Channel Blockers and Risk of Primary Open-angle Glaucoma. *J Glaucoma [Internet].* 2018 Oct 22; Available from: <http://dx.doi.org/10.1097/IJG.0000000000001118>
 41. Mayama C. Calcium channels and their blockers in intraocular pressure and glaucoma. *Eur J Pharmacol.* 2014 Sep 15;739:96–105.
 42. Höhn R, Mirshahi A, Nickels S, Schulz A, Wild PS, Blettner M, et al. Cardiovascular medication and intraocular pressure: results from the Gutenberg Health Study. *Br J Ophthalmol.* 2017 Dec;101(12):1633–7.
 43. Khawaja AP, Chan MPY, Broadway DC, Garway-Heath DF, Luben R, Yip JLY, et al. Systemic Medication and Intraocular Pressure in a British Population. *Ophthalmology.* 2014;121(8):1501–7.
 44. Klein BEK. Intraocular pressure and systemic blood pressure: longitudinal perspective: the Beaver Dam Eye Study. *Br J Ophthalmol.* 2005;89(3):284–7.
 45. Ho H, Shi Y, Chua J, Tham Y-C, Lim SH, Aung T, et al. Association of Systemic
-

- Medication Use With Intraocular Pressure in a Multiethnic Asian Population. *JAMA Ophthalmol.* 2017;135(3):196.
46. Foster PJ, Khawaja AP. The Association of Systemic Medication and Disease With Intraocular Pressure. *JAMA Ophthalmol.* 2017 Mar 1;135(3):203–4.
 47. Hughes AD, Stanton AV, Jabbar AS, Chapman N, Martinez-Perez ME, McG Thom SA. Effect of antihypertensive treatment on retinal microvascular changes in hypertension. *J Hypertens.* 2008 Aug;26(8):1703–7.
 48. Hirooka K, Shiraga F. Potential role for angiotensin-converting enzyme inhibitors in the treatment of glaucoma. *Clin Ophthalmol.* 2007 Sep;1(3):217–23.
 49. Fletcher EL, Phipps JA, Ward MM, Vessey KA, Wilkinson-Berka JL. The renin-angiotensin system in retinal health and disease: Its influence on neurons, glia and the vasculature. *Prog Retin Eye Res.* 2010 Jul;29(4):284–311.
 50. Bowe A, Grünig M, Schubert J, Demir M, Hoffmann V, Kütting F, et al. Circadian Variation in Arterial Blood Pressure and Glaucomatous Optic Neuropathy--A Systematic Review and Meta-Analysis. *Am J Hypertens.* 2015 Sep;28(9):1077–82.
 51. Slagter SN, van Waateringe RP, van Beek AP, van der Klauw MM, Wolffenbuttel BHR, van Vliet-Ostaptchouk JV. Sex, BMI and age differences in metabolic syndrome: the Dutch Lifelines Cohort Study. *Endocr Connect.* 2017 May;6(4):278–88.
 52. Tulassay Z, Stolte M, Sjölund M, Engstrand L, Butruk E, Malfertheiner P, et al. Effect of esomeprazole triple therapy on eradication rates of *Helicobacter pylori*, gastric ulcer healing and prevention of relapse in gastric ulcer patients. *Eur J Gastroenterol Hepatol.* 2008 Jun;20(6):526–36.
 53. Kang JH, Willett WC, Rosner BA, Buys E, Wiggs JL, Pasquale LR. Association of Dietary Nitrate Intake With Primary Open-Angle Glaucoma: A Prospective Analysis From the Nurses' Health Study and Health Professionals Follow-up Study. *JAMA Ophthalmol.* 2016 Mar;134(3):294–303.
 54. Nouri-Mahdavi K, Hoffman D, Coleman AL, Liu G, Li G, Gaasterland D, et al. Predictive factors for glaucomatous visual field progression in the Advanced Glaucoma Intervention Study. *Ophthalmology.* 2004 Sep;111(9):1627–35.
 55. Chauhan BC, Mikelberg FS, Balaszi AG, LeBlanc RP, Lesk MR, Trope GE, et al. Canadian Glaucoma Study: 2. risk factors for the progression of open-angle glaucoma. *Arch Ophthalmol.* 2008 Aug;126(8):1030–6.
 56. Kass MA, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol.* 2002 Jun;120(6):701–13; discussion 829–30.
 57. Gordon MO, Kass MA. The Ocular Hypertension Treatment Study: design and baseline description of the participants. *Arch Ophthalmol.* 1999 May;117(5):573–83.
 58. Keltner JL, Johnson CA, Quigg JM, Cello KE, Kass MA, Gordon MO. Confirmation of visual field abnormalities in the Ocular Hypertension Treatment Study. *Ocular Hypertension Treatment Study Group.* *Arch Ophthalmol.* 2000 Sep;118(9):1187–94.

8

SUMMARY AND GENERAL DISCUSSION

8.1 SUMMARY

The overall aim of this thesis was to investigate the two leading theories of glaucoma and to give insight into how parts of the body other than the eye may be influential in the context of these two theories. The first section of the thesis is devoted to the mechanical theory. In chapter 2, we aimed to determine if glaucoma patients had a lower intracranial pressure (ICP) than controls, and if this difference could be measured noninvasively. A lower ICP has been proposed as one of the backbones of the mechanical theory of glaucoma, and has been shown in a few large studies.^(1–3) We tested this by using a noninvasive method of ICP measurement, called distortion product otoacoustic emissions (DPOAEs), in patients and controls in different body positions. Our findings indicated that there was no evidence that glaucoma patients had a reduced ICP, which agreed with some other recent studies.^(4,5) More broadly, we found that DPOAEs could successfully be used to measure dynamic changes in ICP such as diurnal fluctuations or changes induced by certain medications.

The findings from the first study therefore led us to chapters 3 and 4, which address ICP changes induced by a glaucoma medication called acetazolamide (AAZ). This drug is given to glaucoma patients because it lowers intraocular pressure (IOP) but it also has a known ICP lowering effect.⁽⁶⁾ If the mechanical theory is true, and it is the difference between the two pressures that matters, then a drug that simultaneously lowers both pressures may not be effective. While there are many studies that describe the effect of AAZ on IOP, there are only a few studies that explain its effect on ICP.^(7–9) The aim of chapter 3 was to summarize the available literature on this topic, and to see if it was possible to determine the effect of AAZ on both IOP and ICP by comparing effects at similar doses. The only dose at which there was a crossover in available research for changes in IOP and ICP was 500 mg. At this dose, within 2-6 hours there was an IOP and ICP reduction of 6.2 mmHg (24.7%) and 7.4 mmHg (31.5%), respectively. Therefore at this dosage and time frame, the trans-lamina cribrosa pressure difference (TLCPD) would remain essentially unchanged. We argued that the limited evidence summarized here suggests that AAZ may not be effective for glaucoma — assuming it is the TLCPD that matters, and not IOP alone.

While the information in chapter 3 was a good starting point, the crossover dose of 500 mg is higher than what most glaucoma patients are normally prescribed. In chapter 4, we aimed to test the effect of AAZ on IOP and ICP in the dose most commonly given to glaucoma patients at our clinic (125 mg, twice daily). To do this, we measured IOP and noninvasive ICP (measured with DPOAEs) in glaucoma patients before taking AAZ and for 2 hours post-ingestion, and made comparisons with 13 age-similar controls. We found that after 12 hours with no anti-glaucoma medication, AAZ did not further reduce IOP in glaucoma patients compared to controls ($P=0.58$) but did reduce ICP compared to controls ($P=0.035$), by approximately 4 mmHg. In normal physiological conditions, the pressure from the eye is slightly higher than the pressure from the brain (or retrobulbar space). Our findings suggest that there are periods during the day when this pressure difference is even more pronounced in the case of AAZ use.

The second section of this thesis is devoted to the vascular theory of glaucoma. Since our noninvasive test of ICP in chapter 2 was measured through the ear, many patients asked if their tinnitus would affect the measurement. The complaints seemed to be more numerous than would be expected and so this anecdotal evidence led us to investigate the potential relationship between glaucoma and tinnitus further.⁽¹⁰⁾ In chapter 5, we tested this by first completing a clinic-based cross-sectional questionnaire study in which we sent a series of tinnitus-related questions to glaucoma patients and healthy subjects, and then followed up with a large population-based cross-sectional study in which glaucoma and tinnitus were also assessed by questionnaire. Our results showed that in both studies, those with glaucoma were more likely to have tinnitus than those without glaucoma, even after controlling for various factors associated with both diseases such as: age, gender, BMI, hypertension, diabetes, socioeconomic status, and hearing loss.

In chapter 5 we proposed a mechanism by which glaucoma and tinnitus could be related, namely impaired production of nitric oxide (NO). In chapter 6, we aimed to test this theory by measuring biomarkers of NO and excitotoxicity in glaucoma patients and controls both with and without tinnitus. To do this, serum levels of amino acids associated with NO production were assessed in patients with primary open angle glaucoma (POAG). There was no significant difference in NO biomarker concentrations between glaucoma and controls. Presence of tinnitus also did not significantly affect the biomarkers. However, there was a trend for increased arginine (ARG) in glaucoma patients compared to controls, especially in those with tinnitus. We therefore concluded that glaucoma patients may have a build-up of ARG in the plasma due to a downstream deficiency in the NO production pathway.

Finally, in chapter 7 we aimed to determine the association of statins, antihypertensive medication, and proton pump inhibitors with POAG progression and conversion of POAG suspects to POAG. To do this, we retrospectively investigated the records of a cohort with POAG cases and suspects. Progression and conversion were analyzed with quantile and logistic regression, respectively, with the systemic medications as predictors, controlling for age, gender, BMI, pre-treatment IOP, corneal thickness, and baseline glaucoma stage, depicted by the mean deviation (MD) of the visual field. No systemic medications were associated with POAG progression in the final model. ACE-inhibitors were significantly associated with a decrease in POAG suspect conversion, as were angiotensin II receptor blockers.

8.2 CLINICAL IMPLICATIONS

It is obvious that finding the pathophysiological cause of glaucoma would be beneficial to all patients. However, this thesis was written with two groups of patients in mind; namely, those with normal tension glaucoma (NTG) and those who continue to progress despite having a controlled IOP. In both of these groups, the traditional explanation of a high IOP causing the vision loss in glaucoma is not sufficient.

One of the current leading explanations for these two groups is that they may have a low ICP, however, according to the work presented in chapter 2, both patients with the

“traditional” high IOP glaucoma and NTG patients did not appear to have a lower ICP than healthy subjects. It may be the case that glaucoma patients do not have a constant lower ICP, but that small fluctuations in the TLCPD during the day or while sleeping may contribute to mechanical stress on the lamina cribrosa. In this way, small, transient changes to the TLCPD — like those that may be induced by AAZ, shown in chapters 3 and 4 — may be harmful for glaucoma patients. Although there is not sufficient evidence at this time for clinicians to completely forego AAZ as a treatment option, it may be advisable to more explicitly manage the timing of doses. For instance, research has shown that the TLCPD is higher when seated than when supine. In humans the increase in TLCPD from supine to seated is +8 mmHg and in other primates +14 mmHg.(4,11) If it was deemed to be safe for IOP levels, it may be advantageous to give AAZ only at night before bed, so that patients will be supine during the peak effect of the drug. Another possible treatment in the future could be using drugs that *slightly* increase ICP. For instance, hormonal therapies have been shown to increase ICP and also to prevent glaucoma.(12,13) A possible connection between hormones and ICP could be investigated in the future to determine if it could be a glaucoma treatment option for women. Characterization of continuous, 24-hour changes in both IOP and ICP could lead to better guidance on administration of AAZ and also to the possibility of using drugs that slightly increase ICP in the treatment of glaucoma.

Measuring ICP continuously for 24 hours requires a noninvasive measurement tool. There are many different noninvasive techniques that are still under development, but the key is to determine the best one based not only on accuracy of ICP readings, but also on cost, speed, and operator dependency. For example, ultrasound measurement of optic nerve sheath diameter (ONSD) is commonly used to determine if there is an expansion of the area around the optic nerve due to increased ICP. While this test is cost effective, it is most accurate in those with very high ICP (above 15 mmHg), which hinders its use in glaucoma patients.(14) MR-ICP, which utilizes MRI to predict ICP based on an elastance index, seems to currently be the most accurate measure of ICP over a wide range of pressures.(14,15) However, this method is costly and would also not be ideal for 24-hour monitoring. Hence, in this thesis we discuss DPOAEs as a method for noninvasive ICP measurement. DPOAEs are low cost, easy to use for continuous measurements, and are not operator dependent. A handheld DPOAE device would be easy for clinicians to implement even in hospital or surgical settings. However, the limitation of DPOAEs is that there is a subject-specific cut-off, and so only changes in ICP (and not absolute pressure) can be measured. Testing of DPOAEs while simultaneously measuring invasive ICP could yield actual estimates of absolute ICP in the future.

In regard to the vascular theory and the relationship between tinnitus and glaucoma demonstrated in chapter 5, we do not yet know if the diseases occur simultaneously or if one could be a predictor of the other. In either case, those attending clinics for tinnitus could automatically be referred for a glaucoma screening, or at the very least answer some questions to help determine if a formal screening is warranted (ex: family history of glaucoma and visual complaints from the NEI-VFQ-25 [see section 5.2.2]). Patients attending glaucoma clinics could easily answer a tinnitus questionnaire (see Table 5.2). While tinnitus is not currently

treatable, those with tinnitus who wished to be referred to a specialist could then have access to psychological counseling to deal with the emotional repercussions. Additionally, if glaucoma clinics in areas all around the world were to have data on tinnitus frequency, researchers could build a better understanding of the relationship between the two diseases and of what extrinsic and intrinsic factors may be influential.

More research needs to be done to confirm if, in fact, NO production impairment is a common mechanism that could connect glaucoma and tinnitus, this idea has potential future consequences for diagnosis and treatment. Blood tests could contribute an objective measurement to the subjective diagnosis of tinnitus. Regarding treatment, NO donating medication in the form of an eyedrop is already approved for the treatment of glaucoma for its IOP lowering effect.(16) Possible treatments for oxidative stress and excitotoxicity in both diseases could come in the form of coenzyme Q10 (CoQ₁₀) or GLU receptor antagonists. (17–19) Additionally, patients' diets could be targeted by encouraging a higher intake of nitrate-rich vegetables.(20)

In regard to systemic medication use and the vascular theory, despite some evidence to suggest that statins may be helpful in prevention of glaucoma,(21) we did not confirm this protective effect (Chapter 7). While we did find a protective effect of two antihypertensive medications (angiotensin II receptor blockers [ARBs] and angiotensin-converting enzyme inhibitors [ACEIs]) on POAG suspect conversion, there was a limitation of small sample sizes for those medications. It therefore cannot be recommended at this time that statins, ARBs, or ACEIs be given as a treatment for glaucoma. Conversely, no systemic medications should be discontinued in glaucoma patients because no negative effects were found on progression or suspect conversion.

8.3 RECOMMENDATIONS FOR FUTURE RESEARCH

Mechanical Theory

- Beyond glaucoma, DPOAEs can be used to monitor ICP changes noninvasively, which may improve the understanding of ICP variation and regulation tremendously.
- Future research should focus on a robust and reproducible DPOAE probe placement in order to minimize variability.
- A randomized control trial to determine the effect of AAZ on glaucoma progression would take many years and require a large number of subjects and is therefore unlikely to happen. Therefore a comprehensive understanding of the effect of acetazolamide on ICP and of the TLCPD theory of glaucoma are crucial. Determination of an accurate noninvasive method for ICP assessment is essential to facilitate studies with lower costs and larger sample sizes.
- Measurement of IOP and ICP both with and without a wash-out period of AAZ are needed. Additionally, results with other doses and dosing schedules, and results in glaucoma patients not taking AAZ are needed to better determine if it is helpful or harmful in the treatment of glaucoma.

Vascular Theory

- Future research should focus on the possibility that vascular dysregulation due to impairment of NO production — from both genetic expression and diet — could potentially lead to both glaucoma and tinnitus.
- The chronology of the two diseases should also be characterized with longitudinal studies. Do they occur simultaneously? Or does one come before the other? If the latter is true, then one disease may be a predictor of the other.
- Looking at genes involved in the production of NO in glaucoma patients with and without tinnitus may yield an underlying reason for why these changes occur in the first place.
- Further research could also explore the relationships between glaucoma, tinnitus, and other diseases that have been associated with aberrant NO behavior, specifically diabetes, dementia, and sleep apnea. Investigation of these relationships could lead to an improvement in quality of life for the patients and a better understanding of the pathophysiology of both glaucoma and tinnitus.
- Further investigations, ideally RCTs, are needed to examine the relationships between statins and glaucoma progression and between ARBs and ACEIs and glaucoma suspect conversion.

8.4 HIGHLIGHTS

- Distortion product otoacoustic emissions can be used to measure variations in intracranial pressure noninvasively. (Chapter 2)
- Patients with glaucoma do not have a lower intracranial pressure than controls. (Chapter 2)
- Long-term use of acetazolamide may decrease intracranial pressure transiently while eye pressure remains steady, this could potentially be harmful to glaucoma patients. (Chapter 3 and 4)
- People with glaucoma are more likely to have tinnitus than those without glaucoma suggesting a potential common etiological mechanism. (Chapter 5)
- Slightly elevated plasma arginine levels in glaucoma and tinnitus suggest there may be a downstream defect in NO production (Chapter 6)
- Statins did not have an effect on glaucoma progression or conversion. (Chapter 7)
- ACE-inhibitors and angiotensin II receptor blockers (classes of antihypertensives) both showed a protective effect against conversion from a glaucoma suspect to a diagnosis. (Chapter 7)

REFERENCES

1. Berdahl JP, Fautsch MP, Stinnett SS, Allingham RR. Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. *Invest Ophthalmol Vis Sci.* 2008;49: 5412–5418.
2. Berdahl JP, Allingham RR, Johnson DH. Cerebrospinal fluid pressure is decreased in primary open-angle glaucoma. *Ophthalmology.* 2008;115: 763–768.
3. Ren R, Jonas JB, Tian G, Zhen Y, Ma K, Li S, et al. Cerebrospinal fluid pressure in glaucoma: a prospective study. *Ophthalmology.* 2010;117: 259–266.
4. Lindén C, Qvarlander S, Jóhannesson G, Johansson E, Östlund F, Malm J, et al. Normal-Tension Glaucoma Has Normal Intracranial Pressure: A Prospective Study of Intracranial Pressure and Intraocular Pressure in Different Body Positions. *Ophthalmology.* 2018;125: 361–368.
5. Pircher A, Remonda L, Weinreb RN, Killer HE. Translaminar pressure in Caucasian normal tension glaucoma patients. *Acta Ophthalmol.* 2017;95: e524–e531.
6. Supuran CT. Acetazolamide for the treatment of idiopathic intracranial hypertension. *Expert Rev Neurother.* 2015;15: 851–856.
7. Chaaban MR, Illing E, Riley KO, Woodworth BA. Acetazolamide for high intracranial pressure cerebrospinal fluid leaks. *Int Forum Allergy Rhinol.* 2013;3: 718–721.
8. Gücer G, Viernstein L. Long-term intracranial pressure recording in the management of pseudotumor cerebri. *J Neurosurg.* 1978;49: 256–263.
9. Smith SV, Friedman DI. The Idiopathic Intracranial Hypertension Treatment Trial: A Review of the Outcomes. *Headache: The Journal of Head and Face Pain.* 2017. pp. 1303–1310. doi:10.1111/head.13144
10. Konieczka K, Choi HJ, Koch S, Fankhauser F, Schoetzaue A, Kim DM. Relationship between normal tension glaucoma and Flammer syndrome. *EPMA J.* 2017;8: 111–117.
11. Jasien JV, Samuels BC, Johnston JM, Downs JC. Effect of Body Position on Intraocular Pressure (IOP), Intracranial Pressure (ICP), and Translaminar Pressure (TLP) Via Continuous Wireless Telemetry in Nonhuman Primates (NHPs). *Invest Ophthalmol Vis Sci.* 2020;61: 18.
12. Tan MG, Worley B, Kim WB, Ten Hove M, Beecker J. Drug-Induced Intracranial Hypertension: A Systematic Review and Critical Assessment of Drug-Induced Causes. *Am J Clin Dermatol.* 2020;21: 163–172.
13. Newman-Casey PA, Talwar N, Nan B, Musch DC, Pasquale LR, Stein JD. The potential association between postmenopausal hormone use and primary open-angle glaucoma. *JAMA Ophthalmol.* 2014;132: 298–303.
14. Price DA, Grzybowski A, Eikenberry J, Januleviciene I, Verticchio Vercellin AC, Mathew S, et al. Review of non-invasive intracranial pressure measurement techniques for ophthalmology applications. *Br J Ophthalmol.* 2020;104: 887–892.
15. Alperin NJ, Lee SH, Loth F, Raksin PB, Lichtor T. MR-Intracranial pressure (ICP): a method to measure intracranial elastance and pressure noninvasively by means of MR imaging: baboon and human study. *Radiology.* 2000;217: 877–885.

16. Garhöfer G, Schmetterer L. Nitric oxide: a drug target for glaucoma revisited. *Drug Discov Today*. 2019;24: 1614–1620.
17. Imsuwansri T, Hoare DJ, Phaisaltuntiwongs W, Srisubat A, Snidvongs K. Glutamate receptor antagonists for tinnitus. *Cochrane Database of Systematic Reviews*. 2016. doi:10.1002/14651858.cd012391
18. Sharma DK, Kaur S, Singh J, Kaur I. Role of acamprosate in sensorineural tinnitus. *Indian J Pharmacol*. 2012;44: 93–96.
19. Doozandeh A, Yazdani S. Neuroprotection in Glaucoma. *J Ophthalmic Vis Res*. 2016;11: 209–220.
20. Kang JH, Willett WC, Rosner BA, Buys E, Wiggs JL, Pasquale LR. Association of Dietary Nitrate Intake With Primary Open-Angle Glaucoma: A Prospective Analysis From the Nurses' Health Study and Health Professionals Follow-up Study. *JAMA Ophthalmol*. 2016;134: 294–303.
21. McCann P, Hogg RE, Fallis R, Azuara-Blanco A. The Effect of Statins on Intraocular Pressure and on the Incidence and Progression of Glaucoma: A Systematic Review and Meta-Analysis. *Invest Ophthalmol Vis Sci*. 2016;57: 2729–2748.

Appendices

Nederlandse samenvatting

Acknowledgments

NEDERLANDSE SAMENVATTING

Het algemene doel van dit proefschrift was om de twee leidende theorieën van glaucoom te onderzoeken en om inzicht te geven in hoe andere delen van het lichaam dan het oog, invloed kunnen hebben in de context van deze twee theorieën. Het eerste deel van het proefschrift is gewijd aan de mechanische theorie. In hoofdstuk 2 was het doel te bepalen of glaucoompatiënten een lagere intracraniele druk (ICP) hadden dan gezonde controles, en of dit verschil niet-invasief kon worden gemeten. Een lagere ICP is gesuggereerd als het achterliggende fundament van de mechanische theorie van glaucoom, en is aangetoond in enkele grote studies. [1–3] We hebben dit getest door een niet-invasieve methode van ICP-meting te gebruiken, genaamd *distortion product otoacoustic emissions* (DPOAE's), bij patiënten en controles in verschillende lichaamshoudingen. Onze bevindingen gaven aan dat er geen bewijs was dat glaucoompatiënten een lagere ICP hadden, wat overeenkwam met enkele andere recente onderzoeken. [4,5] In een bredere context, we vonden dat DPOAE's met succes kon worden gebruikt om dynamische veranderingen in ICP te meten, zoals dagelijkse fluctuaties of veranderingen veroorzaakt door bepaalde medicijnen.

De bevindingen van de eerste studie leidden ons daarom naar de hoofdstukken 3 en 4, waarin ICP-veranderingen, die veroorzaakt worden door een glaucoommedicijn genaamd acetazolamide (AAZ), worden bestudeerd. Dit medicijn wordt aan glaucoompatiënten gegeven omdat dit de intraoculaire druk (IOP) verlaagt, maar het heeft ook een bekend ICP-verlagend effect. [6] Als de mechanische theorie waar is, en het is het verschil tussen de twee drukken dat ertoe doet, dan is een medicijn dat beide drukken tegelijkertijd verlaagt mogelijk niet effectief. Hoewel er veel studies zijn die het effect van AAZ op IOP beschrijven, zijn er maar een paar studies die het effect ervan op ICP onderzoeken. [7-9] Het doel van hoofdstuk 3 was om de beschikbare literatuur over dit onderwerp samen te vatten, en om te zien of het mogelijk was om het effect van AAZ op zowel IOP als ICP te bepalen door de effecten bij vergelijkbare doses te vergelijken. De enige dosis, in de beschikbare literatuur, waarbij er een cross-over was voor veranderingen in IOP en ICP was 500 mg. Bij deze dosis was er binnen 2-6 uur een IOP- en ICP-reductie van respectievelijk 6,2 mmHg (24,7%) en 7,4 mmHg (31,5%). Daarom zou bij deze dosering en dit tijdsbestek het drukverschil over de lamina cribrosa (TLCPD) ongewijzigd blijven. We stelden dat het beperkte bewijs dat hier is samengevat, suggereert dat AAZ mogelijk niet effectief is voor glaucoom — ervan uitgaande dat het de TLCPD is die ertoe doet, en niet alleen IOP.

Hoewel de informatie in hoofdstuk 3 een goed uitgangspunt was, is de cross-overdosis van 500 mg hoger dan wat de meeste glaucoompatiënten normaal gesproken wordt voorgeschreven. In hoofdstuk 4 was het doel om het effect van AAZ op IOP en ICP te testen in de dosis die het meest wordt gegeven aan glaucoompatiënten in onze kliniek (125 mg, tweemaal daags). Om dit te doen, hebben we IOP en niet-invasieve ICP (gemeten met DPOAE's) bij glaucoompatiënten gemeten voordat AAZ werd ingenomen en gedurende 2 uur na inname, vervolgens hebben we de vergelijking gemaakt met 13 op leeftijd vergelijkbare controles. We vonden dat AAZ na 12 uur zonder anti-glaucoommedicatie, de IOP bij glaucoompatiënten niet verder verlaagde in vergelijking met controles, maar de

ICP wel verlaagde in vergelijking met controles, met ongeveer 4 mmHg. Onder normale fysiologische omstandigheden is de druk van het oog iets hoger dan de druk van de hersenen (of retrobulbaire ruimte). Onze bevindingen suggereren dat er periodes zijn gedurende de dag dat dit drukverschil nog sterker aanwezig is in het geval van AAZ-gebruik.

Het tweede deel van dit proefschrift is gewijd aan de vasculaire theorie van glaucoom. Omdat onze niet-invasieve test van ICP in hoofdstuk 2 werd gemeten via het oor, vroegen veel patiënten of hun tinnitus de meting zou beïnvloeden. De klachten leken talrijker te zijn dan verwacht en dit anekdotisch bewijs bracht ons ertoe de mogelijke relatie tussen glaucoom en tinnitus verder te onderzoeken. [10] In hoofdstuk 5 hebben we dit getest door eerst een klinische cross-sectionele vragenlijststudie af te ronden waarin we een reeks tinnitusgerelateerde vragen naar glaucoompatiënten en gezonde proefpersonen stuurden, en daarna is dit opgevolgd met een groot cross-sectioneel bevolkingsonderzoek, waarin glaucoom en tinnitus ook werden beoordeeld door middel van een vragenlijst. Onze resultaten toonden aan dat in beide onderzoeken degenen met glaucoom meer kans hadden op tinnitus dan degenen zonder glaucoom, zelfs na statistische controle voor verschillende factoren die verband houden met beide ziekten, zoals leeftijd, geslacht, BMI, hypertensie, diabetes, sociaaleconomische status en gehoorverlies.

In hoofdstuk 5 hebben we een mechanisme voorgesteld waarmee glaucoom en tinnitus gerelateerd kunnen worden, namelijk een verminderde productie van stikstofmonoxide (NO). In hoofdstuk 6 was het doel deze theorie te testen door biomarkers van NO en excitotoxiciteit te meten bij glaucoompatiënten en controles zowel met als zonder tinnitus. Om dit te doen, werden serumspiegels van aminozuren die gerelateerd zijn aan NO-productie, gemeten bij patiënten met primair openhoekglaucoom (POAG). De monsters werden ook vergeleken met referentiewaarden van de doelmetabolieten bij gezonde mensen. Er was geen significant verschil in concentraties van NO-biomarkers tussen glaucoompatiënten en controles. De aanwezigheid van tinnitus had ook geen significante invloed op de biomarkers. Er was echter wel een trend voor verhoogde arginine (ARG) bij glaucoompatiënten in vergelijking met controles, vooral bij degenen met tinnitus. We concludeerden daarom dat glaucoompatiënten een opbouw van ARG in het plasma kunnen hebben als gevolg van een defect laat in het productietraject van NO.

Ten slotte was het doel in hoofdstuk 7 het bepalen van de associatie van statines, antihypertensiva en protonpompremmers met de progressie van POAG en conversie van POAG-verdachten naar daadwerkelijk POAG. Hiervoor hebben we retrospectief onderzoek gedaan van de medische dossiers van een cohort met POAG-patiënten en -verdachten. Progressie en conversie werden geanalyseerd met respectievelijk kwantiele en logistische regressie, met de systemische medicatie als voorspellers. Hierbij werd gecontroleerd voor leeftijd, geslacht, BMI, IOP vóór de behandeling, hoornvliesdikte en baseline glaucoomstadium, weergegeven door de gemiddelde deviatie (MD) van het gezichtsveld. In het uiteindelijke model was geen systemische medicatie geassocieerd met POAG-progressie. ACE-remmers waren significant geassocieerd met een afname van POAG-verdachte conversie, evenals angiotensine II-receptorblokkers.

REFERENCES

1. Berdahl JP, Fautsch MP, Stinnett SS, Allingham RR. Intracranial pressure in primary open angle glaucoma, normal tension glaucoma, and ocular hypertension: a case-control study. *Invest Ophthalmol Vis Sci*. 2008;49: 5412–5418.
 2. Berdahl JP, Allingham RR, Johnson DH. Cerebrospinal fluid pressure is decreased in primary open-angle glaucoma. *Ophthalmology*. 2008;115: 763–768.
 3. Ren R, Jonas JB, Tian G, Zhen Y, Ma K, Li S, et al. Cerebrospinal fluid pressure in glaucoma: a prospective study. *Ophthalmology*. 2010;117: 259–266.
 4. Lindén C, Qvarlander S, Jóhannesson G, Johansson E, Östlund F, Malm J, et al. Normal-Tension Glaucoma Has Normal Intracranial Pressure: A Prospective Study of Intracranial Pressure and Intraocular Pressure in Different Body Positions. *Ophthalmology*. 2018;125: 361–368.
 5. Pircher A, Remonda L, Weinreb RN, Killer HE. Translaminar pressure in Caucasian normal tension glaucoma patients. *Acta Ophthalmol*. 2017;95: e524–e531.
 6. Supuran CT. Acetazolamide for the treatment of idiopathic intracranial hypertension. *Expert Rev Neurother*. 2015;15: 851–856.
 7. Chaaban MR, Illing E, Riley KO, Woodworth BA. Acetazolamide for high intracranial pressure cerebrospinal fluid leaks. *Int Forum Allergy Rhinol*. 2013;3: 718–721.
 8. Gücer G, Viernstein L. Long-term intracranial pressure recording in the management of pseudotumor cerebri. *J Neurosurg*. 1978;49: 256–263.
 9. Smith SV, Friedman DI. The Idiopathic Intracranial Hypertension Treatment Trial: A Review of the Outcomes. *Headache: The Journal of Head and Face Pain*. 2017. pp. 1303–1310. doi:10.1111/head.13144
 10. Konieczka K, Choi HJ, Koch S, Fankhauser F, Schoetzau A, Kim DM. Relationship between normal tension glaucoma and Flammer syndrome. *EPMA J*. 2017;8: 111–117.
-
-

ACKNOWLEDGEMENTS

This thesis would not have been possible without the love, support, and knowledge of countless people. From the previous scientific work it is built upon, to the emotional support and conversations which stimulated new ideas, to the publication and design of the book itself. In this way, at times I feel I am only the public relations manager of this thesis. But in my capacity in that role, there are a few specific people I would like to thank.

Nomdo, Pim, and Emile, you gave me the freedom to explore my own ideas and yet the guidance and advice to make all of those ideas come to fruition. You have truly made me an independent scientist and I hope to work with you again someday in the future.

Mom and Dad, it's hard to sum up in a few sentences a "thank you" for making me the person I am. But, thank you for raising me with a sense of curiosity about the world, a lack of limits to what I could achieve, and a foundation of love and kindness I can only hope to emulate. Brova, looking up to you has always made me strive to be a more creative and insightful person. Your unique insights into the world and people around you has made my scientific mind more astute.

To the people of EGRET(+), spending the last four years learning and growing with you has been a pleasure. Kim, you are the glue that held it all together and I hope you know how invaluable you are. Stephen, thank you for reigniting my enthusiasm for science and for always believing in me, I'm not sure how I would have survived 2020 without you. Konstantinos, your intelligence pushed me to work harder, and your friendship and jokes helped me through times of self-doubt more than you'll ever know. Iris, I was too awkward to get to know you until well into the PhD but I'm so glad I did. Your dedication to learning new things and enthusiasm for science was a breath of fresh air, and your friendship remains one of the best outcomes from my time in Groningen.

To the Ballers, I still can't believe I was lucky enough to find you crazy people. My transition into adulthood was so drastically affected by your friendship. Your inquisitive minds, senses of humor and adventure, and unconditional love have made me a better person. No matter how spread out we are geographically, I always hold you all in my heart. Mason, if it wasn't for you, I wouldn't have the Ballers, the Netherlands, or this PhD. You were on the frontlines of the doubts and insecurities and you supported me through it all. You have made me see people and the world from a place of kindness and compassion and I will be forever grateful for that.