Home Blood Pressure in Health and Disease

Peder af Geijerstam
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Abstract

Hypertension is the most common preventable cause of premature all-cause mortality, primarily from cardiovascular disease (CVD). Individuals with dysglycemia, including prediabetes and diabetes, are at increased risk. Licorice intake raises blood pressure (BP) through the effects of glycyrrhizic acid (GA), but the true limit of safe intake is uncertain. Home BP has several benefits over BP measured at a clinic, including a higher predictive value for CVD. By combining office and home BP, it is possible to diagnose masked hypertension (MH), in which home but not office BP is elevated, and white coat hypertension (WCH), in which office but not home BP is elevated. The aim of this thesis was to advance our knowledge on home BP in relation to dysglycemia, markers of CVD, and licorice intake.

The first 3 papers used data from the Linköping cohort of the prospective Swedish CArdioPulmonary bioImage Study (SCAPIS). Study IV was a randomized controlled cross-over study. Data was obtained from questionnaires, blood samples and office and home BP measurements. In studies I-III, pulse wave velocity (PWV), coronary artery calcium score (CACS), and carotid artery plaques as markers of CVD were also included.

In Study I, we examined 5025 men and women aged 50-64 years old for the relation between dysglycemia and home BP. Both the systolic office and home BP measurements were positively associated with dysglycemia. Participants with dysglycemia vs normoglycemia more often had MH. The findings were in line with previous research and strengthened the association between dysglycemia and MH.

In Study II, we examined the associations between MH and markers of CVD in 4122 individuals without BP-lowering treatment. Of participants, 4.2% had MH, and these were more often men and had higher BMI than those with normotension.
Participants with MH also had higher odds for CACS ≥100, an association which has previously been suggested as a trend.

In Study III, we examined the relation between soluble P-selectin (sP-selectin) as a measure of thrombotic activity, plasma high-sensitivity C-reactive protein (hsCRP) as a measure of inflammation, and home BP in 4548 participants. Both markers were higher in each hypertension phenotype compared with sustained normotension. The quartile of participants with the highest sP-selectin values had higher odds for CACS ≥100 and carotid artery plaques. The association between sP-selectin and sustained hypertension was novel and not affected by adjustments for hsCRP.

In Study IV, 28 healthy participants aged 18-30 years old were evaluated for the effects of a daily intake of licorice containing 100 mg of GA compared with a control product for 2 weeks. During the licorice intake period, the systolic home BP increased with 3.1 mmHg, and the suppression of serum aldosterone and plasma renin levels indicated that this was due to the licorice intake.

In conclusion, this thesis further strengthens the idea that both home and office BP measurements have values beyond that of the other, and that home BP may be most valuable in individuals with dysglycemia and obesity, and in men. Finally, licorice may be more potent than previously known, suggesting the need for increased awareness.
Svensk sammanfattning

Förhöjt blodtryck (hypertoni) är den huvudsakliga orsaken till forntida död, främst genom hjärtkärlsjukdom. Flera mekanismer och riskfaktorer som kan förklara hypertoni har identifierats. Individer med förhöjt blodsocker, inklusive diabetes och dess förstadier, löper ökad risk för hjärtkärlsjukdom, och förhöjt blodsocker samexisterar ofta med hypertoni. Lakritsintag höjer blodtrycket genom dess beståndsdel glycyrrhizinsyra (GA), och även om både Europeiska unionen och Världshälsoorganisationen har föreslagit att ett intag av upp till 100 mg per dag sannolikt är säkert att förätta för de flesta individer, är den gränsnivån osäker.

Jämfört med blodtryck som mäts på mottagningen av medicinsk utbildad personal har hemblodtryck flera fördelar, inklusive starkare koppling till framtida hjärtkärlsjukdom. Genom att kombinera mottagnings- och hemblodtryck går det att diagnosticera maskerad hypertoni (MH), då hemblodtrycket är förhöjt trots normalt mottagningsblodtryck, och vitrockhypertoni (WCH), då mottagningsblodtrycket men inte hemblodtrycket är förhöjd. Syftet med denna avhandling var att vidare utforska hemblodtryck i relation till förhöjt blodsocker, markörer för hjärtkärlsjukdom, och lakritsintag.

Avhandlingens fyra arbeten baseras på två studier. I de första tre arbetena analyserades data från Linköpings-kohorten i the Swedish CArdioPulmonary bioImage Study (SCAPIS), en prospektiv studie av män och kvinnor i åldrarna 50-64 år. I det sista arbetet analyserades data från en lottad överkorsningsstudie. Data i studierna bestod av enkätsvar, blodprover, samt mottagnings- och hemblodtrycksmätningar. I studie I-III ingick även pulsvågshastighet, kalkhalt i kranskärlen vid skiktröntgen (CACS) och plackförekomst i halspulsådrorna vid ultraljudsundersökning som markörer på hjärtkärlsjukdom.

I det första delarbetet undersökte vi data för 5025 individer avseende blodtryck och förhöjt blodsocker. Både mottagnings- och
Hemblodtryck var associerat med förhöjt blodsocker. Deltagare med förhöjt blodsocker hade oftare MH jämfört med de med normalt blodsocker, och skillnaden mellan mottagnings- och hemblodtrycket var omvänt associerat med långtidsblodsocker. Fynden var i linje med tidigare forskning, och stärkte kända kopplingar mellan MH och förhöjt blodsocker. Förklaringarna till detta samband är okända, men möjliga sådana inkluderar selektiva effekter av blodtryckssänkande läkemedel hos individer med förhöjt blodsocker, samsjukligheter såsom fetma, och aktivering av det sympatiska nervsystemet.

I det andra delarbetet analyserade vi förekomst av och associationer för MH hos 4122 individer utan pågående blodtryckssänkande läkemedelsbehandling. Av dessa hade 4.2% MH, och dessa var oftare män och hade högre BMI än de med normalt blodtryck. Deltagare med MH hade också högre pulsvågshastighet och oftare förhöjt CACS. Associationen mellan MH och markörer för hjärtsjukdom var tidigare känd och styrktes av våra resultat.

I det tredje delarbetet undersökte vi 4548 deltagare avseende två blodprovsmarkörer: en för blodplättsaktivitet, lösligt P-selektin i blodet, och en för inflammation, högkänsligt C-reaktivt protein (hsCRP). Både P-selektin och hsCRP var högre vid hypertoni, oavsett typ, jämfört med vid normalt blodtryck. Den kvartil av deltagarna som hade högst P-selektin hade oftare WCH och hypertoni både hemma och på mottagningen, jämfört med normalt blodtryck, och oftare förhöjt CACS och plack i halspulsådrorna. Associationen mellan P-selektin och högt blodtryck både hemma och på mottagningen var inte tidigare känd, och påverkades inte av justering för hsCRP, vilket antydde att den inte endast förklarades av inflammation.

I det fjärde och sista delarbetet inkluderas 28 friska individer i åldrarna 18 till 30 år. I en överkorsningsstudie bad vi deltagarna att dagligen under 2 veckor inta antingen lakrits med ett innehåll av 100 mg GA eller en kontrollprodukt utan lakrits. Deltagarna

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undersöktes med avseende på hemblodtryck, liksom hormonnivåer i blodet. Under lakrits- jämfört med kontrollperioden steg det systoliska hemblodtrycket med 3,1 mmHg, och hormonnivåerna påverkades på ett sätt som talade för att GA påverkade blodtrycket.

Sammanfattningsvis stärker studiernas resultat kunskapen om att blodtrycksmätning både på mottagningen och i hemmet är värdefullt både var för sig och tillsammans, och att hemblodtryck är särskilt värdefullt hos individer med förhöjt blodsocker eller övervikt, samt hos män. Slutligen visade sig små mängder lakrits påverka kroppen mer än tidigare känt, och ökad medvetenhet och bättre etikettering av lakritsprodukter kan vara befogad.
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Peder af Geijerstam
List of Papers


### Abbreviations

<table>
<thead>
<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>CACS</td>
<td>Coronary artery calcium score</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>GA</td>
<td>Glycyrrhizic acid</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated hemoglobin</td>
</tr>
<tr>
<td>MH</td>
<td>Masked hypertension</td>
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<tr>
<td>PWV</td>
<td>Pulse wave velocity</td>
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<tr>
<td>RAAS</td>
<td>Renin-angiotensin-aldosterone system</td>
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<tr>
<td>SCAPIS</td>
<td>The Swedish CArdioPulmonary bioImage Study</td>
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<tr>
<td>WCH</td>
<td>White coat hypertension</td>
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<td>WHO</td>
<td>World Health Organization.</td>
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Introduction

Hypertension

Hypertension is the leading preventable cause of premature all-cause mortality, primarily by cardiovascular disease (CVD) including ischemic heart disease and stroke (1, 2). Around 30-45% of the global population is affected by hypertension, and the number is increasing (2). In Sweden, most individuals with hypertension are managed in primary care (3). Treating hypertension reduces morbidity and mortality (2), but only a minority of individuals are diagnosed and sufficiently treated (4).

Hypertension is defined as elevated blood pressure (BP) according to specific criteria, but elevated BP does not always equal hypertension. Blood pressure is increased physiologically in several circumstances when tissue demand for oxygenated blood is increased. For example, systolic BP can increase up to 3-fold during heavy isotonic resistance training (5, 6), in which the compression of veins and respiratory maneuvers also affect BP (6-8). During weight lifting, systolic BP as high as 480 mmHg has been recorded (5). During aerobic exercise, systolic BP can increase by around 40-60%, from normotensive to between 170 to 220 mmHg (9-11). In fact, although high systolic BP during and after exercise is associated with incident hypertension and increased cardiovascular risk (12-14), during maximum aerobic exercise, a low rather than a high peak systolic BP is associated with increased mortality (14). Similarly, in a study of 119 151 individuals admitted with chest pain, higher admission systolic BP was associated with lower mortality at follow-up (15).

Thus, it is not primarily momentarily elevated BP that is harmful, except in extreme cases in susceptible individuals (6). Rather, it is the sustained, unrelenting hypertension, and the
consequences it has on the arterial system, tissues, and vital organs such as the kidneys, heart, and brain, that is harmful. To understand these processes and effects is key to understand the importance of hypertension.

A Brief History

Early descriptions of an illness akin to hypertension date back to the 10th century or perhaps even before that, but understanding was limited (16, 17). In China, accounts of a harder or stiffer pulse as a result of excessive salt intake were made as far back as 1700 BC (18, 19). In 1733, Stephen Hales was able to measure BP for the first time, invasively in horses, but it took until the mid-19th century before the first non-invasive measuring device was introduced, enabling recordings on humans (1). The term *l'hypertension artérielle* was used by Henri Huchard in 1893 (20).

By the turn of the 20th century, the inflatable bladder was introduced and the auscultatory method was discovered (1). Around this time, the association between elevated BP, end-organ damage, and increased mortality was suggested (1). Despite this, notions that perhaps the high BP was compensatory and necessary for perfusion persisted into the 1940s (21), and it wasn’t until the 1950s that effective treatments, some of which are still in use today, emerged (1). However, into the 1990s, trials and thus treatments were aimed at higher BP levels, and the current more strict treatment targets have only been used for the past few decades (21).

The Cardiac Cycle and Vascular Resistance

The physiology of the cardiovascular system, and thus BP, is complex. The arteries transport blood from the heart to the tissues and organs, and arterial BP is determined by the cardiac output and the resistance of the vasculature (22, 23). The cardiac output is the stroke volume times the heart rate (24). In systole, the left ventricle
contracts to eject blood, and thus produces the upper or systolic BP; in diastole, the left ventricle relaxes to refill with blood from the venous system, and the BP that is maintained is the lower or diastolic BP (22).

In the young and healthy, some of this systolic blood volume and pressure is absorbed by the elastic, proximal arteries, and is then released during diastole when these arteries recoil (25). This function contributes to a lower systolic and a higher diastolic BP, and thus a more even pressure during the cardiac cycle (2, 25, 26). With age, arteries lose some of this elasticity, a phenomenon termed arterial stiffness. This causes an increase in systolic BP, a decrease in diastolic BP, and thus increased pulse pressure, which can be used as a crude indicator of arterial stiffness (2, 22, 25). In fact, arterial stiffness as a risk factor for CVD has a longer history than BP itself, initially studied with tactile sphygmology (Greek sphygmos = pulse, -logia = study of) (26). Aortic stiffening impedes the even flow of blood through the vascular tree, and the relative increase in the systolic BP explains why isolated systolic hypertension becomes more common with age (22, 27). Aortic stiffening can also predict future development of hypertension (27). Finally, in individuals with hypertension, arterial stiffness can be persistent because of vascular damage, and/or momentary because of increased arterial wall tension as a result of the elevated BP (28).

In summary, the cardiac cycle, the diastolic and systolic BP, the elasticity of the arteries, and the development of CVD, are intrinsically linked.

Pathophysiology

In about 5-10% of individuals with hypertension, a specific cause can be identified (29, 30). Of the most common are renovascular (e.g., renal artery stenosis) and endocrine diseases (e.g., primary
hyperaldosteronism), as well as obstructive sleep apnea (30). This is referred to as secondary hypertension.

In most cases, however, the cause is much more complex and not yet fully understood, and this is referred to as primary hypertension (1, 29). Mechanisms may include inflammation, the gastrointestinal microbiome, and renal regulation, resulting from environmental and lifestyle factors such as alcohol, smoking, stress and licorice intake (which is discussed in detail in a dedicated chapter), as well as ageing and the arterial stiffness previously mentioned (1, 2, 29, 31). More than 1000 genetic factors have also been identified, including several affecting the renal salt regulation and the renin-angiotensin-aldosterone system (RAAS) (2, 29).

Obesity, dyslipidemia, and diabetes are also linked to high BP. Although obesity is associated with hypertension, adults with both obesity and hypertension have elevated BP but not body weight during childhood (32). One theory is that elevated insulin levels could cause both dyslipidemia and play a central role in the development of hypertension, the latter through effects on smooth muscle, sodium retention, and/or stimulation of the sympathetic nervous system (32, 33). In type 1 diabetes, insulin levels are depleted. In type 2 diabetes, however, insulin levels are often increased at the beginning of the disease (34), and insulin resistance, which characterizes type 2 diabetes, is associated with incident hypertension (35). Insulin resistance and insulin secretion are also associated with increased pulse wave velocity (PWV), a marker of vascular ageing (36, 37). However, a causal relationship between insulin and hypertension is uncertain (33, 38), and in a study of 596 individuals, insulin levels were not associated with the future development of hypertension (32).

Central to hypertension, however, is the hemodynamic characteristics of increased systemic vascular resistance, and the RAAS (2). The RAAS is one of the primary regulators of BP and includes some of the most prominent vasoactive substances with effects on
Introduction

Salt and water balance as well as arterial vasoconstriction (22, 29, 39). Renin is a hormone secreted by the kidneys in response to low BP, hyponatremia and signaling from the sympathetic nervous system (39). Angiotensinogen, secreted by the liver, is hydrolyzed by renin into angiotensin I, which is in turn converted by the angiotensin-converting enzyme (ACE) into angiotensin II (39). This hormone acts as a vasoconstrictor and stimulates sodium and water reabsorption in the kidneys, thus increasing BP, and also stimulates secretion of aldosterone from the adrenal glands (22, 39). Aldosterone acts on the kidneys to retain sodium and waste potassium (39). RAAS also acts on the central nervous system to stimulate thirst and secrete antidiuretic hormone, which depresses diuresis (39). Beyond this, RAAS also affects adiposity, inflammation and dysglycemia, and is in turn also affected by several other hormonal systems, including sex hormones, thyroid hormones, and cortisol (39). In summary, RAAS activity increases BP primarily through vasoconstriction and increased water retention.

Finally, salt intake may be essential to the age-related rise in BP, which has not been seen in communities with little or no salt consumption (18, 19, 40), as exemplified by Joossens in a 1980 publication (41), Figure 1. These studies could methodologically not fully account for all potential confounders such as obesity, physical activity, alcohol use, and stress, but the results were nonetheless striking. To test the 1960 salt hypothesis of Dahl, who first described the association between sodium intake and hypertension prevalence across populations, an international group of researchers conceived the multi-center INTERSALT study in 1982 (42). The study, which included 10 079 individuals of 52 populations and 32 countries, found that sodium excretion was associated with the age-related increase in BP (19, 43). In 4 of the populations, urinary sodium excretion was far below the mean of the other populations whilst minimal or no increases in BP, and no hypertension, were seen with age (43). The Cardiovascular Diseases and
Alimentary Comparison (CARDIAC) Study, which began in 1985, was planned to further evaluate this association (44). The multi-center study included 7550 individuals of 20 countries, and 24-h urinary sodium excretion values were again positively correlated to both systolic and diastolic BP, also after adjustment for body mass index (BMI) (44).

**Figure 1.** Comparative international data on salt intake and blood pressure levels (41), reproduced by kind permission of the Royal Society of Medicine.

Reproduced by kind permission of the Royal Society of Medicine from Joossens JV. Dietary Salt Restriction: The Case in Favour. In: Robertson, Pickering, Caldwell (editors). International Congress and Symposium Series Number 26: The Therapeutics of Hypertension. London: The Royal Society of Medicine; 1980. p. 243-50. Figure 4, Comparative international data on salt intake and blood pressure levels; p. 247.

Studies have since concluded that high dietary sodium intake could explain around 30% of hypertension worldwide (45).
However, most of these studies had a relatively short follow-up time, and the impact of excessive sodium intake may be even greater (45). In a recent review, only 5 of 133 studies had a duration of more than 6 months, and studies of longer duration were called for (46).

The kidneys may be evolutionary more adept to handle low sodium and high potassium (41). The habitual adding of salt to food may have occurred for only the most recent fraction of human history, sparked by the advent of agriculture and the need to preserve food (18, 19). Based on metabolic studies and estimates of the amount of salt consumed in Paleolithic diets and hunter-gatherer societies, it is highly likely that less than 1 gram of sodium per day is physiological, and that a daily intake as low as ¼ gram may be sufficient with only a few rare exceptions (47). However, the maximum recommended intake of most guidelines is 2 grams, and the global daily sodium intake is on average around twice that (4 grams), with all of that excess the result of sodium added to food products during processing, preparation, and serving (45). Physiologically, it is thus estimated that we require somewhere no more than 1/4th, and perhaps as little as 1/16th, of the current average sodium intake (46, 47).

Lowering salt intake also reduces cardiovascular risk and the risk of all-cause mortality, and there is no evidence of a lower threshold at which reduced salt intake would increase risk (29, 40, 48, 49). Results of previous research indicating such a J-shaped relationship may have been affected by reverse causation, confounding, drop-out, or sparse or erroneous measurements (48). Sensitivity to salt intake also varies between individuals, with high baseline BP, known hypertension and older age being associated with higher salt sensitivity, but other factors including genetics may also play a role (45, 49). Primary aldosteronism, with increased aldosterone levels, also increases salt sensitivity, and high aldosterone may also explain increased salt sensitivity in obese
individuals (50). Low potassium intake may further increase BP, especially in individuals with high sodium intake, hypertension, or increased salt sensitivity (51). Finally, to reduce the risk of hypertension, reducing sodium intake may have to begin early in life, as the otherwise elevated BP may lead to irreversible hemodynamic changes (50).

In summary, hypertension is likely caused by an intricate combination of factors, as well as a negative cycle by which initially transient changes and increases in BP may lead to irreversible hemodynamic and anatomical changes (e.g., arterial stiffness as discussed previously) (2, 50).

Classification

Blood pressure is commonly measured as the systolic and diastolic BP and expressed in millimeters of mercury (mmHg). Hypertension is diagnosed when either or both of these are elevated above a certain cut-off, which varies between guidelines and the method through which measurements are obtained. Of the 3 major guidelines on hypertension, both the 2020 International Society of Hypertension (ISH) and the 2023 European Society of Hypertension (ESH) suggest the cut-off 140 over 90 mmHg for office BP, and 135 over 85 mmHg for home and ambulatory BP, while the 2017 American College of Cardiology (ACC) and American Heart Association (AHA) guidelines propose the cut-off 130 over 80 mmHg for both office and home BP (2, 52, 53), Table 1.

These diagnostic thresholds have been determined based on when treatment has reduced cardiovascular risk in trials, but the risk is increased already when the systolic office BP is above 115 mmHg (2, 40, 54). In Sweden, the systolic BP of 18-year-old male conscripts increased gradually from 1969 to 2010 to a mean above 130 mmHg and a hypertension prevalence using the ESH criteria of 32.5% (55). The cardiovascular risk with increased BP is exponential, and is strongly associated with age (40). Treatment targets
partly reflect this difference, as they are set below the diagnostic thresholds, Table 1, but they are still far higher than the level at which the cardiovascular risk is increased (2). Also, more recent studies have challenged these thresholds, as further discussed in the chapter on Treatment.

Table 1. Diagnostic criteria and treatment targets for office, home, and ambulatory BP in mmHg in individuals with hypertension aged 18–79 years according to guidelines (2, 52, 53).

<table>
<thead>
<tr>
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<th>Diagnostic thresholds</th>
<th>Treatment targets</th>
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<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
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<tr>
<td><strong>Office measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESH and ISH</td>
<td>≥140</td>
<td>≥90</td>
</tr>
<tr>
<td>ACC/AHA</td>
<td>≥130 a</td>
<td>≥80 a</td>
</tr>
<tr>
<td><strong>Home measurements or ambulatory daytime measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESH and ISH</td>
<td>≥135</td>
<td>≥85</td>
</tr>
<tr>
<td>ACC/AHA</td>
<td>≥130</td>
<td>≥80</td>
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<tr>
<td><strong>Ambulatory nighttime measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESH and ISH</td>
<td>≥120</td>
<td>≥70</td>
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<tr>
<td>ACC/AHA</td>
<td>≥110</td>
<td>≥65</td>
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<tr>
<td><strong>Ambulatory 24-hour measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESH and ISH</td>
<td>≥130</td>
<td>≥80</td>
</tr>
<tr>
<td>ACC/AHA</td>
<td>≥125</td>
<td>≥75</td>
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In the respective guidelines, home and ambulatory BP values were either specified as criteria and target thresholds, indicated as equivalent to the corresponding office BP values, or indicated as equivalent to the corresponding grade of hypertension. Treatment targets in the ESH Guidelines are applicable to patients aged 65–79 years only if well tolerated. Treatment targets in the ISH Guidelines are applicable only if tolerated, and for patients aged ≥65 years, target BP is <140 systolic and <90 diastolic.

a. If not ≥140 systolic or ≥90 diastolic, and 10-year CVD risk <10%, initial nonpharmacological treatment is recommended.
b. In ESH guidelines, home measurements are reasoned to be “a few mmHg” below the corresponding office BP values.

Abbreviations: ACC, American College of Cardiology; AHA, American...
Complications

Hypertension is the leading cause of mortality in the world, and it affects more than 1 billion people (52). It often leads to organ damage, some of which will be discussed here. The relationship between higher systolic BP and increased cardiovascular mortality is continuous, and there is no clear evidence of a lower BP threshold at which the risk of ischemic heart disease would increase with lower BP (56). Thus, no set threshold of risk, as could be perceived by those used for diagnosis and treatment titration, exists (57).

In the heart, hypertension can cause coronary atherosclerosis which increases the risk of ischemic heart disease, conduction disorders, atrial fibrillation, heart failure, and aortic syndromes (2, 40). The risk of coronary heart disease is increased via several mechanisms. Arterial stiffness leads to increased vascular resistance and thus pulsatile afterload, which can cause heart failure as well as increased oxygen demand for the myocardium (58-60). Oxygen is supplied to the heart via the coronary arteries, which emanate from the most proximal aorta and thus receive most of their blood flow during diastole (61). The decreased diastolic pressure in individuals with arterial stiffness impedes this blood flow to the coronary arteries, and the increased workload and decreased oxygen supply may cause ischemic heart disease (61). In fact, although increased diastolic pressure is an independent risk factor for CVD (62), increased pulse pressure may be a more negative prognostic factor for ischemic heart disease such that for a given systolic BP, lower diastolic BP is associated with an increased risk of coronary heart disease (63), Figure 2. Increased BP may also favor both plaque formation and plaque disruption, and left ventricular hypertrophy and endothelial dysfunction may also contribute (64).
**Figure 2.** Diastolic blood pressure and the hazard ratio for coronary heart disease depending on systolic blood pressure categories (63).

Reproduced by kind permission of Wolters Kluwer Health, Inc. From Franklin, S. S. et al (1999). Is pulse pressure useful in predicting risk for coronary heart Disease? The Framingham heart study. *Circulation*, 100(4), 354–360. Doi: https://doi.org/10.1161/01.cir.100.4.354. *Circulation* is published by the American Heart Association. The Creative Commons license does not apply to this content. Use of the material in any format is prohibited without written permission from the publisher, Wolters Kluwer Health, Inc. Please contact permissions@lww.com for further information. Abbreviations: CHD, coronary heart disease; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Hypertension may also be the most important risk factor for heart failure, mainly as diastolic dysfunction, but also, at least with concomitant ischemic heart disease, as systolic dysfunction (65). In hypertension, diastolic dysfunction is caused by volume and pressure overload, leading to hypertrophy and subsequent dysfunctional filling and distention of the left ventricle (65). Left
ventricular hypertrophy may also be exacerbated by increased RAAS activation, stimulated by stress to the left ventricle itself (65).

Hypertension also increases the risk of chronic kidney disease, for which only diabetes is a more important risk factor (2). Furthermore, high BP affects the central nervous system, both acutely in terms of ischemic and hemorrhagic stroke, but also silently with changes that can eventually lead to the development of cognitive decline and dementia (2). Markers of vascular ageing, including PWV and pulse pressure, are also associated with worse cognitive performance both cross-sectionally and prospectively, but causality is uncertain (2). Finally, hypertension may also cause retinopathy, retinal vascular disease, and optic neuropathy because of ischemia (2), as well as peripheral artery disease (66).

**Treatment**

Reducing the BP in hypertension reduces the risk of both heart failure and ischemic heart disease (65). Akin to the discrepancies between the diagnostic criteria of hypertension in different guidelines, treatment targets also vary, both for office, home, and ambulatory BP, Table 1 (2, 52, 53).

In a 2016 meta-analysis including 123 studies and 613,815 individuals, each 10 mmHg reduction of systolic BP reduced incident cardiovascular events by 20% and all-cause mortality by 13% (67). Effects were seen even when baseline systolic BP was below 130 mmHg (67). A more recent 2022 meta-analysis of 48 trials and 344,716 individuals found, similarly, that a reduction of systolic BP by 5 mmHg reduced the risk of cardiovascular events by 10%, but also that the risk reduction was seen even when the initial systolic BP was below 120 mmHg (68). Reflecting on the current standard of treating hypertension based on whether BP is elevated above specific thresholds or not, the authors concluded that the decision may rather be based on assessments which also include other...
cardiovascular risk factors (68). Other studies, too, have suggested a reduced focus on set thresholds in hypertension treatment (57).

Home Blood Pressure

In the advent of home BP measurements, it was referred to as a method to diagnose white coat hypertension (WCH), and to identify individuals at risk of hypotensive side effects of BP-lowering treatment, rather than other purposes such as to detect hypertension at home despite normal office BP (69). Several early studies on measuring BP at home in the 1970s, looking at for example compliance and effects on BP, used either separate sphygmomanometers and stethoscopes, or sphygmomanometers with built-in stethoscopes (70-72). Later, portable devices used microphones to detect the Korotkoff sounds and then print these signals on paper, or ultrasound probes to detect equivalent signals (73-76), Figure 3. Although these devices were only semiautomatic, they enabled ambulatory self-measurements through-out entire days (76).

The oscillometric technique was discovered in 1876, but it took another century for it to be more widely adapted (77). In the 1970s, the automated oscillometric BP measuring device, which is still in use today, was introduced, and there are now thousands of models available (1, 78). Measurements are performed by placing a cuff around the upper arm, after which the cuff is inflated and then deflated whilst a pressure sensor inside the cuff measures blood vessel oscillations (1, 79, 80). “Oscillometric”, however, is a misnomer, as the device captures pressure waveforms rather than oscillations (78). Through algorithms, the systolic and diastolic BPs are then estimated, and the accuracy of such estimates are verified using validation protocols (22, 77, 78). Occasionally, the term “automated” also denotes devices which measure BP at pre-programmed intervals rather than at the discretion of the user (81).
**Figure 3.** Portable blood pressure recorder of the Remler Company, California, USA (76).

In the clinic, automated BP monitoring is more accurate than manual BP measurements, when compared against ambulatory BP measurements (82). Automated recorders also decrease user-dependent variations, including digit preference (83), and are recommended for both office and home BP (77, 84). However, compared to invasive measurements of BP, automated vs auscultatory
measurements underestimate systolic BP to a somewhat larger degree (78).

Automated devices made it possible for home BP measurements to become more widespread and easy for patients to adopt, and also made possible ambulatory BP monitoring, whereby BP is measured automatically at certain intervals for a full 24 hours (79). Collectively, home and ambulatory BP are referred to as out-of-office BP (2, 79). The distinction between home and ambulatory BP monitoring devices is becoming less clear, as several home BP devices may also measure night-time BP using timers, with comparable results in terms of both absolute values, the ratio between BP at night and during the day, and associations with target organ damage (85).

Early automated recorders using microphones or dopplers weighted between 8 and 14 kg and carried a cost of almost 4000 USD after conversion to current value, but current devices using the oscillometric technique weight less than 1 kg and often cost less than 100 USD (83, 86). This combination of technological advance and improved portability and affordability has enabled the increased use of home BP.

**Current Use and Research Interest**

In current guidelines, out-of-office BP is regarded as significant in the diagnosis of hypertension (52, 53). The American College of Cardiology recommends out-of-office BP both when diagnosing hypertension and when titrating BP-lowering medication (53). The International Society of Hypertension suggests that elevated office BP should always be confirmed with out-of-office BP, except in their minimum standards of care recommendation (52). The European Society of Hypertension recommends out-of-office BP when WCH or masked hypertension (MH) is suspected, in follow-up, and when feasible to confirm the diagnosis of hypertension (2).
The interest in home BP has also increased over time, and the number of publications per 100 000 in the PubMed database has more than tripled in the last 3 decades to 179 publications in 2023. At the same time, the relative number of publications on BP in general has more than halved (87), Figure 4.

**Figure 4.** The relative number of publications on PubMed with the search term “blood pressure” per 0.1 million total publications shown as solid triangles and a dashed trend line, and “home blood pressure” per 1 million (sic!) total publications shown as solid circles and a solid trend line, produced using PubMed by Year (87).

**Reproducibility and Costs**

The reproducibility of measurements, defined as the precision in measurements over time or occasions, is an important aspect in both research and clinical practice (88). Home BP has better reproducibility short-term (weeks apart) vs both ambulatory and
office BP, as well as long-term (year over year) (89, 90). The better reproducibility of home vs office BP has been suggested as valuable in predicting BP levels as far out as 3 years from the initial measurements (32).

Blood pressure that is elevated in the initial recordings often falls during subsequent measurements, which has previously been attributed to an “alarm reaction” which is reduced with both time and repeated visits (91). However, although this has not been refuted, much of the variation in BP over time is now thought to be explained by the regression to the mean phenomenon (92). The degree of this regression is lower for out-of-office vs office BP measurements, possibly because the error from the true mean is reduced when measurements are repeated, or because higher baseline values are associated with greater reductions over time according to the law of initial value (92-97). In home BP, regression to the mean is most pronounced in the first 3 months after the initial measurement but continues to influence results to a lesser degree for at least another 12 months (92). In both home and ambulatory BP, regression is upward for individuals with low baseline readings, and downward for those with high baseline readings (92-94), Figure 5. This may have clinical consequences in terms of misclassification, as the categorization of measurements as normotensive or hypertensive may fluctuate considerably over time, especially for individuals with high baseline readings (92).

Finally, a 2023 systematic review of 16 studies found out-of-office vs office BP monitoring to be more cost-effective in relation to BP reduction or quality-adjusted life-years. This was especially true for time periods of at least 10 years. Ambulatory BP, however, was even more cost-effective than home BP (98).

In summary, out-of-office vs office BP has several benefits, with regards to labor-intensity, costs, accessibility, and reproducibility.
Figure 5. Regression to the mean for home and office BP measurements, stratified according to baseline BP levels (92).

Participants are stratified according to baseline BP levels, for systolic <120, 120-129, 130-139, 140-149, and ≥150 mmHg; for diastolic <70, 70-79, 80-89, and ≥90 mmHg.

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Abbreviations: DBP, diastolic blood pressure; mmHg, millimeters of mercury; SBP, systolic blood pressure.

Use in Research

Some of the above benefits of home BP measurements are also relevant in the research context. Home BP may be used in trials as a means to follow BP amongst participants in a less labor-intensive and more accessible way (31, 99), with reduced costs and improved convenience for study participants. Furthermore, because of the
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better reproducibility and less pronounced regression to the mean of home BP, it may increase the power of clinical trials (100). This effect could, as discussed, be explained by the repeated measurements, a lesser regression to the mean, and a lesser white coat effect, as well as reduced digit preference and bias of the observer than what is seen with office BP measurements (100). Interestingly, reproducibility for ambulatory BP may be less than for home BP despite repeated measurements, perhaps because of the stricter conditions applied to home BP (100). However, home BP devices used in research are validated, but a majority of those marketed online may be non-validated and at only about half the cost of the validated devices (101), and the effects of such conditions on reliability in the clinical setting is unknown.

Finally, random measurement error is the random fluctuation of measurements around the true value, as opposed to systematic error when the error does not average to zero. Random measurement error can cause regression dilution bias i.e., an attenuated association to the dependent variable because of a flattened regression slope, and this is also reduced with measurements based on more numerous readings, such as home BP (102).

Comparing Office and Out-Of-Office Blood Pressure

By combining office and out-of-office BP, it is possible to diagnose intermediate hypertension phenotypes: WCH and MH (103), Table 2. Masked hypertension is defined as normal office BP, but high out-of-office BP, and WCH is defined as high office BP, but normal out-of-office BP. When both BP measurements are normal, the term “sustained normotension” is used, and when both are elevated, the term “sustained hypertension” is used. The difference between the office and home BP is termed the “white coat effect” (or sometimes “office effect”) when the office BP is higher, which is the most common (104-106), and the “masked effect”, “home effect”, or “reverse white-coat effect” when the home BP is higher.
(106-108). The term white coat effect rather than WCH is also advocated by some for individuals with BP-lowering treatment and WCH, as they by definition have an underlying hypertension nonetheless (109). Also, the “white coat condition” has alternatively been used to describe elevated BP in the presence of medical staff (110). Thus, to describe the numeric difference between the office and home BPs, the office-home BP difference (or office to out-of-office BP difference, when including ambulatory BP measurements), is a more neutral and specific terminology and one which will be used henceforth in this thesis.

**Table 2.** Classification of blood pressure status based on office and out-of-office blood pressure measurements.

<table>
<thead>
<tr>
<th>Out-of-office blood pressure</th>
<th>Office blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Sustained normotension</td>
</tr>
<tr>
<td>High</td>
<td>Masked hypertension</td>
</tr>
<tr>
<td></td>
<td>Sustained hypertension</td>
</tr>
</tbody>
</table>

Classification of WCH and MH depends on the definition of normal office and out-of-office BP, respectively. When categorizing BP measurements as normal or elevated, current guidelines use thresholds for home BP that are 5 mmHg lower than the office BP counterpart, regardless of age and sex, Table 1. In studies, the definition of a significant white coat effect is more heterogenous e.g., a systolic office BP at least 10 mmHg higher than the home BP (111), a systolic office BP at least 20 mmHg higher than the home BP (112), or as the highest tertile of office-home BP difference (106). Depending on these thresholds, the distribution and characteristics of participants between groups differ.

Associations for the systolic office-home BP difference have been assessed based on different measurements (home BP in the morning and/or evening, varying number of days, etc) and with
different definitions of the office-home BP difference (as a continuous variable, as tertiles, or otherwise categorized), but in most studies it decreases with age, in men, with higher BMI, in individuals with diabetes, in cigarette smokers (current and/or previous), and in individuals with BP-lowering medication (106, 107, 113-115). Finally, there are relatively few studies on the white coat effect using home BP measurements in primary care (111).

The pathophysiology behind the office to out-of-office BP difference is not entirely clear, but it may at least partly be explained by the regression to the mean phenomenon discussed earlier, which is more pronounced for office vs out-of-office BP measurements. It has also been attributed to anxiety or an “alarm reaction” caused by the clinic visit itself (91, 110, 116-118). This effect may be reduced with repeated measurements during single visits or over several visits, more relaxing clinic environments, and biofeedback treatment (91, 116, 119, 120). Individuals with greater white coat effect may also have a more pronounced BP reaction to public speaking (117). Also, the inverse association with cigarette smoking may be explained by reduced anxiety because of nicotine (121). Other studies have seen no association between individuals with WCH and anxiety, and suggest that anticipation of a negative result i.e., a high BP, is the culprit (110). Individuals that are informed of their high BP at the clinic have higher BP during subsequent clinic measurements compared with individuals with equally high baseline BP that are not informed (118). Such a reaction may become long-lasting as a conditioned reflex, even when the actual fear of a negative result is no longer present (110), but it is not known whether this awareness response is also present in out-of-office BP. The response may be mediated by sympathetic or neuro-endocrine stimuli (110), and the white coat effect is associated with increased plasma cortisol levels (121).
Factors Related to Patients

For individuals living with CVD, home BP may increase their sense of empowerment and involvement in their disease management (122). Although home BP may also trigger stress, it can provide reassurance for patients, and is associated with better adherence to BP-lowering medication and increased hypertension control rates (123-125). However, health literacy is an important factor in the use of home BP (126), and self-reported BP values, as with other data such as blood sugar in individuals with diabetes, may be inaccurate because of manipulation or human error (127).

In the Diagnosis and Treatment of Hypertension

For diagnosing hypertension, a systematic review of 20 studies up until 2010, including 5863 individuals, showed insufficient sensitivity and specificity of either office or home BP alone when compared to ambulatory BP (128). However, such a comparison assumes that ambulatory BP would record the “true” BP, which may have merits in theory but for which there is no clear evidence. Also, no studies have compared treatment of hypertension diagnosed using home vs office BP, so the significance thereof is yet unknown.

Hypertension thresholds for home and office BP were evaluated in a 2014 meta-analysis of 5018 individuals from 5 populations. The study confirmed the current standards of using the same BP threshold regardless of age and sex, as no such differences were found. However, in individuals without BP-lowering medication, a systolic office vs home BP of 140 vs 130-131 mmHg and 130 vs 122-124 mmHg carried equal 10-year cardiovascular risk, thus corresponding to a slightly larger difference between the systolic office and home BP of 6-10 mmHg compared to the standard difference of 5 mmHg used in current guidelines (54). However, with lower BP, the difference between office and home BP decreases (2), and when treated to a systolic BP target below 130 mmHg, mean office and daytime ambulatory BP may differ by less than 1 mmHg (129).
Although the difference may be small on a population level, the difference may be greater at an individual level, and the benefit of targeting home vs office BP reduction has not been sufficiently studied. A randomized controlled trial comparing BP-lowering treatment initiation and titration towards office and ambulatory BP measurements vs only home BP measurements found no difference in changes of left ventricular mass index, PWV, albuminuria, home or ambulatory BP values, or hypertension control rates (130). However, the follow-up time was only a little more than 1 year, and effects on cardiovascular events and mortality were not assessed (130). In a randomized controlled trial comparing titration of BP-lowering medication to a home BP of <135 mmHg systolic and <85 mmHg diastolic vs <125 mmHg systolic and <80 mmHg diastolic, evaluating more than 3000 participants during a median follow-up of 5.3 years, no difference was seen in incident MACE (131). However, a gradual decrease in cardiovascular risk was seen with decreased home BP, which led the authors to conclude that a systolic home BP target of <130 mmHg should be safe (131).

In the 2023 European Society of Hypertension guidelines for the management of arterial hypertension, the paucity of large randomized controlled trials on treatment targeting home BP is considered a “crucial gap” (2).

**Cardiovascular Risk**

Compared with office BP, out-of-office BP has several benefits, including better predicting cardiovascular mortality (132, 133). For predicting cardiovascular events, neither of home nor ambulatory BP is superior to the other (103, 123, 134). Compared with office BP, home BP is also better correlated to end-organ damage, including left ventricular hypertrophy, carotid atherosclerosis, silent cerebrovascular disease, stroke, and end-stage renal disease (79, 123).
In a systematic review of 8 prospective cohort studies up until February 2023, BP measurements derived from home BP monitoring were associated with increased risk of CVD after multiple adjustments, including office BP, with a hazard ratio of 1.30 and 1.21 for systolic and diastolic BP, respectively. Similar results were seen for ambulatory BP (135). However, there was heterogeneity, indicating potential methodological and/or clinical diversity, for results on systolic home but not ambulatory BP (135).

**Follow-up Using Home Blood Pressure**

In a systematic review and meta-analysis including 6522 participants with hypertension and at least one comorbidity, BP self-monitoring using home BP compared with usual care reduced systolic office BP with 3.1 mmHg and reduced the likelihood of uncontrolled hypertension at follow-up (136). In another systematic review of 18 studies up until 2023, of which 9 were randomized controlled trials, use of home BP monitoring improved systolic BP in patients with chronic kidney disease (137). In the majority of these studies, patients received thorough education on how to use and measure BP at home, and many times also follow-up sessions to ensure adequate use (137), thus perhaps exceeding the quality of the usual care given today with regards to home BP monitoring. In a meta-analysis of 9 studies including 1061 participants between 1992 and 2015, systolic and diastolic ambulatory BP was reduced with 3.64 and 2.16 mmHg, respectively, when hypertension was treated to home vs office BP targets (138).

Combined with telemonitoring, using home BP may be even more beneficial. A systematic review and meta-analysis of 6 randomized controlled trials between 2000 and 2022, including a total of 1550 patients, compared the effects of pharmacist-led home BP telemonitoring to usual care after a period of between 5 and 12 months (139). Home BP with telemonitoring, which was performed via telephone, video call or cell phone applications,
reduced systolic and diastolic BP with 8.09 and 4.19 mmHg, respectively, compared with usual care in which BP was measured by health care professionals at the office (139). In a systematic review and meta-analysis of 39 randomized controlled trials, including a total of 23,952 participants, home BP telemonitoring vs usual care reduced systolic and diastolic office BP with 3.99 and 2.00 mmHg, respectively (140). Finally, a 2011 meta-analysis of 12 randomized controlled trials on home BP telemonitoring vs usual care, including more than 4000 participants with a follow-up of between 8 and 240 weeks, showed that the use of BP-lowering medications increased, office and ambulatory BP decreased, and the proportion with normalization of office BP increased (141). Home BP may also reduce therapeutic inertia in resistant hypertension (142). However, how much of the BP-lowering effect that can be attributed to the increased level of care and the contact with health care professionals, vs the use of home BP, is not clear.

Finally, in pregnancy, meta-analyses of up to 5335 pregnant women have shown that the use of home vs office BP monitoring may reduce some adverse outcomes, but several outcomes were also unaffected and one of the reviews concluded that methodology varied too much between studies to allow for any clear conclusion or guidance (143, 144). In the postpartum period, a systematic review concluded that home BP probably improves BP reporting and reduces racial disparities in adherence to BP monitoring, and that it might reduce admissions rates related to hypertension (145).

White Coat Hypertension

Depending on the definition used and cohort studied, white coat hypertension (WCH) prevalence ranges between 10 and 50% (117), and is more common with older age and in women (121). WCH increases the risk of sustained hypertension (117). Several studies, mainly using ambulatory BP as the method for out-of-office BP, have found associations between WCH and markers of CVD.
A meta-analysis of 25 cross-sectional studies including 7382 participants evaluated with office and ambulatory BP showed that echocardiographic measurements of left ventricular mass and diastolic dysfunction were worse in those with WCH vs sustained normotension, yet better than in participants with sustained hypertension (146). Another cross-sectional study of 139 individuals without BP-lowering medication found that more novel echocardiographic measurements of systolic function were also worse in individuals with WCH vs sustained normotension (147). Individuals with WCH also have higher C-reactive protein levels, which in turn is associated with arteriosclerosis (148-150).

Several meta-analyses of cross-sectional studies using office and ambulatory BP have also reported associations between WCH and higher PWV and intima-media thickness. In a meta-analysis of 20 studies, including 5120 adult participants both with and without BP-lowering medications, individuals with WCH had higher PWV than those with sustained normotension (151). In another meta-analysis of studies including 2352 participants without BP-lowering medications, those with WCH had higher PWV vs those with sustained normotension, but not different from those with MH (152). Finally, in a meta-analysis of 25 studies including 8100 individuals, PWV, intima-media thickness, and left ventricular mass index were again between those of sustained normotension and sustained hypertension (153). In a meta-analysis of 10 studies including 3478 participants without BP-lowering medication, the intima-media thickness of the common carotid artery increased gradually from those with normotension, through WCH, to individuals with sustained hypertension (154).

As described above, several studies have found associations between WCH and markers of CVD, but out-of-office compared with office BP is more strongly correlated to end-organ damage and cardiovascular events. Whether WCH increases the risk of future cardiovascular events has been a long-standing topic, and data
from randomized controlled trials is insufficient (22, 69, 117). In a meta-analysis of 14 observational studies, including 29 100 participants followed for a mean of 8 years, the risk of cardiovascular events and mortality increased from those with sustained normotension, through WCH, to sustained hypertension (155). However, the risk of stroke and all-cause mortality was not different between those with sustained normotension and WCH (155). In a meta-analysis of 4 prospective cohort studies, including 5954 individuals with or without BP-lowering medication evaluated using office and ambulatory BP and followed for a median of 5.4 years, results were inconclusive as to whether WCH increased the risk of stroke or not (156). In a study of 7295 individuals without diastolic hypertension or previous CVD, evaluated with office and ambulatory BP, the risk of cardiovascular events during a median follow-up time of 10.6 years was similar between those with WCH and sustained normotension, regardless of use of BP-lowering medication (157). In a 2022 prospective cohort study of 1423 subjects without baseline organ damage, evaluated using office and ambulatory BP, WCH compared with sustained normotension was independently associated with incident MH or sustained hypertension and end-organ damage after 10 years, and increased cardiovascular mortality, but not all-cause mortality, after a median follow-up of 29 years (158). In a registry study of 59 746 individuals evaluated with office and ambulatory BP on clinical (and not study) grounds, and followed for a median of 9.7 years, masked and sustained hypertension, but not WCH, were associated with increased risk of all-cause and cardiovascular mortality (159). In fact, WCH was associated with a decreased risk of all-cause death, HR 0.90 (159). However, this may have been due to an inverse association between diastolic office BP and these endpoints, and when participants with diastolic office BP below 70 mmHg were excluded, those with WCH had similar risk as those with sustained normotension (159).
In 2 different meta-analyses, each with studies including a total of almost 5000 women, the risk of complications in pregnancy, including preeclampsia and preterm birth, were increased in participants with WCH vs sustained normotension (160, 161).

Finally, the reproducibility of WCH is limited. In a study of 153 mostly women participants, the correlation between the white-coat effect at baseline and after 4 years was moderate, \( r = 0.41 \), and decreased slightly with time. A high white-coat effect at baseline was associated with a larger decrease of the white-coat effect at 4 years, and vice versa, indication some regression to the mean. The white-coat effect variation over time was largely attributed to variation in the office BP (162).

In summary, WCH may be associated with increased risk of CVD and thus not a completely benign entity, but data is somewhat conflicting.

**Treatment**

It has been speculated that the increased cardiovascular risk and effects on cardiac systolic function associated with WCH may be attributed to sympathetic nervous system activation, rather than the BP itself, and that treatment therefor may not attenuate the risk (104, 153). However, studies have evaluated effects on BP rather than incident CVD and mortality. In a study on the effects of BP-lowering treatment, retrospectively analyzing a randomized controlled trial, both office and ambulatory BP were reduced in those with sustained hypertension, but only office BP (and not ambulatory BP) was reduced in those with WCH (163). Similar results were seen in a 2014 analysis of a randomized controlled trial including 1921 individuals with hypertension (164).

The European Society of Hypertension suggests managing WCH with lifestyle changes, and only consider BP-lowering medication if additional cardiovascular risk factors are present (2).
Masked Hypertension

Masked hypertension is defined as a high out-of-office BP despite a normal office BP, thus hidden or “masked” from the clinician if only the office BP is evaluated (165). This condition was historically referred to as “reverse WCH”, “hypertension at home”, “isolated home hypertension”, or “undetected ambulatory hypertension”, but Pickering et al coined the current terminology in 2002 (166-168). Parenthetically, “masked hypertension” had been used to describe the phenomena of low BP after myocardial infarction as early as 1978 (169). Masked hypertension can be diagnosed using either home or ambulatory BP, but as many as 30% of individuals only have MH when one or the other of these 2 methods is used and would thus not be diagnosed using only one of the methods (170). Depending on methodology and cohort characteristics such as sex distribution, age, and co-morbidities, prevalence varies between 5.2-29.5% (121, 168, 171-175), and may also vary as much as 2-fold depending on the criteria used (173).

When out-of-office BP is higher, it could be an issue of a less pronounced stress-response to the office setting, and/or any factor that may selectively increase out-of-office BP, including smoking and sympathetic overactivity (176). Cross-sectionally, individuals with MH have higher 24-hour urinary epinephrine levels than those with normotension or other hypertension phenotypes (177). However, daytime stress may affect office and out-of-office BP equally (113). Also, in a 2007 study, 56 individuals with hypertension had higher muscle sympathetic nerve activity compared with the 20 participants with normotension, but this difference was not dependent on hypertension phenotype (178).

For individuals with hypertension, the difference may also be due to either morning office BP measurements coinciding with peak effects of medications, and/or increased adherence to medications ahead of physician visits (“white coat adherence”) (179). BP-lowering medications also reduce office BP on average
50% more than ambulatory BP (179). However, such factors cannot explain MH in individuals without BP-lowering medications.

The morning BP surge, which in theory affects half of all home BP registrations, may also contribute to the higher home vs office BP, MH, and the increased risk it is associated with (180). BP decreases by 10-20% during sleep, and then surges in the morning with a peak after waking (181, 182). Exogenous melatonin reduces BP (183), but it is not known whether this effect is mediated by improved sleep quality or via other mechanism. Circadian BP rhythm (i.e., endogenous 24-hour oscillations, as opposed to diurnal rhythms, which are environmental, triggered by external input such as light), however, affects BP mainly in the evening, and the least during the morning, and does not seem to be associated with other well-known circadian variations such as cortisol levels (184). Thus, the morning surge in BP may be explained by other factors. The morning surge in individuals with hypertension and CVD is more pronounced, which could be due to sympathetic nervous system activation, RAAS activation, plasma cortisol levels and/or arterial stiffness (185, 186). This morning surge in BP also coincides with a peak in cardiovascular events (186) and both a higher morning BP surge and a higher morning vs evening BP is associated with cardiovascular events and mortality (187, 188). In a study of 3303 individuals evaluated using office and home BP measurements, MH was more often diagnosed when using only the morning vs evening home BP measurements, 23.1 vs 14.7% (189). Desaturation during the night can increase both nighttime and morning BP (188), so that obstructive sleep apnea may increase not only ambulatory but home BP as well.

Masked hypertension, regardless of method used for diagnosis, is cross-sectionally associated with smoking, diabetes, and history of CVD (168, 170, 171, 179, 190). A trend, albeit not significant, has been found towards higher prevalence of coronary artery
calcifications (191). Masked hypertension diagnosed using home BP is also associated with older age, higher BMI, higher cholesterol, and increased carotid intima-media thickness (170, 171, 192); MH diagnosed using ambulatory BP is also associated with younger age, obstructive sleep apnea, left ventricular hypertrophy, chronic kidney disease, and BP-lowering medication (168, 170, 179, 190, 193, 194).

In a meta-analysis of 21 prospective observational studies performed before 2019, utilizing either home or ambulatory BP monitoring in 130,318 participants with or without BP-lowering medications, MH vs sustained normotension was associated with an increased risk of all-cause mortality with a risk ratio of 1.67 (195). The risk of cardiovascular mortality was likewise increased, risk ratio 2.19 (195). The risks of cardiovascular and renal disease events were also elevated, risk ratios 1.71 and 3.85, respectively (195). No difference was found between studies using home vs ambulatory BP monitoring (195). In individuals with hypertension, even with office BP levels below 120 mmHg systolic and 80 mmHg diastolic, a 10 mmHg increase in systolic home BP is associated with a hazard ratio of 1.21 for all-cause mortality (196).

Finally, reproducibility of MH classification may be low. In a meta-analysis of 11 and 6 studies, respectively, the reproducibility of MH was 0.41 with ambulatory and 0.26 with home BP, largely attributed to the poor reproducibility of office BP (197). This variability in office BP may be due to different degrees of alerting response between visits (198) or the regression to the mean phenomenon. For ambulatory BP, variations may depend on environmental factors such as stress, sleep, and exercise patterns, as well as treatment adherence which also varies over time (198). Reproducibility may be even worse in clinical practice because of variations in staff, routines, and environment. Also, some previous studies
have been criticized for not using the same BP monitoring device and intervals in the office and at home (199).

Dysglycemia and Diabetes

Dysglycemia is the collective term for when blood glucose levels are higher than normal on repeated measurements, and is divided into prediabetes and diabetes (200). Both have distinct diagnostic criteria based on blood glucose and blood glycated hemoglobin (HbA1c) levels, but is clinically a continuum. The criterias differ slightly between the 2 most recognized guidelines, the WHO and the American Diabetes Association, but Swedish guidelines are based on those of the WHO, Table 3 (201, 202).

Table 3. Diagnostic criteria for prediabetes and diabetes in Sweden, with reference to the World Health Organization guidelines (201).

<table>
<thead>
<tr>
<th>Glycated hemoglobin</th>
<th>Fasting plasma glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemia</td>
<td>&lt;42 mmol/mol</td>
</tr>
<tr>
<td>Prediabetes</td>
<td>42-47 mmol/mol</td>
</tr>
<tr>
<td>Diabetes</td>
<td>≥48 mmol/mol</td>
</tr>
</tbody>
</table>

Several forms of diabetes have been recognized, but the two most common are type 1 and type 2 (203). The former is the result of autoimmune destruction of pancreatic β-cells, which produce insulin, and is characterized by insulin deficiency (203). The vast majority with dysglycemia, however, have type 2 diabetes, which also involves β-cell dysfunction, albeit most often preceded by insulin resistance, which is the hallmark of type 2 diabetes, and in most individuals obesity (203). Both types of diabetes are increasing in prevalence, but type 2 diabetes is the most prevalent (203), and the type to which most studies mentioned henceforth refer.
Introduction

Diabetes increases the risk of CVD 2-fold, and the risk is highest in those with an early diagnosis and poor glycemic control (202). As with hypertension, the increased risk of CVD begins at levels below the diagnostic cut-offs (202). Diabetes also shares several complications with hypertension, including increased risk of both ischemic heart disease, heart failure, atrial fibrillation, and chronic kidney disease (202).

Diabetes and hypertension are intrinsically linked (204). First and foremost, they both increase the risk of CVD independently, and the risk may be higher than each on their own when both occur in the same individual (205). As previously described, hyperinsulinemia in the obese has also been linked (as one of several factors) to the development of hypertension (206, 207). Elevated BP, starting from as low as 110 mmHg systolic, is also associated with an increased risk of diabetes (208). Lowering BP reduces this risk, but whether this risk reduction is explained by the actual reduction of BP, or by the inhibition of RAAS, is debated (208). Thus, causality has not been established, and chronic inflammation, which is present in both diabetes, hypertension, and obesity, could also play a role (204, 208).

Inflammation

Inflammation is a complex and diverse phenomenon which can be both protective, as in the response to an infection, and pathological (209). It is associated with both hypertension and CVD, as well as the development of type 2 diabetes (210-215). Inflammation is a principal component in the formation of atherosclerosis, which leads to narrowing of the arterial lumen and contributes to the development of CVD including ischemic heart disease and stroke (216-218). Individuals with chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis, have an increased risk of CVD (218). Individuals with transient increases in inflammation, for example after receiving vaccinations or during bouts of a chronic
inflammatory disease, have higher PWV (218). Pulse wave velocity also correlates with the degree of inflammation in individuals with chronic inflammatory conditions (218). Several anti-inflammatory medications, such as corticosteroids and TNF-alpha inhibitors, reduce both inflammatory markers and PWV, but may also negatively affect other risk factors for CVD such as dysglycemia and dyslipidemia, and increase incident cardiovascular events (218).

**C-Reactive Protein**

C-reactive protein (CRP) can be measured conventionally or as high-sensitivity CRP (hsCRP), by which lower values can be detected (219). This is one of the most common markers of inflammation, which correlates to obesity, dysglycemia, BP, arterial stiffness, and atherosclerosis (214, 217, 220-222). Levels of hsCRP may also be associated with the development of hypertension and predict future cardiovascular events (218, 222). However, causality between CRP and CVD has not been established, and CRP genotypes, which affect CRP levels, are not associated with neither arterial stiffness nor coronary heart disease (218).

**Soluble P-Selectin**

Selectins are molecules best known for mediating the tethering and rolling of leukocytes (223). This protein family consists of 3 members, each named after the cell type in which they were first found: E-selectin in endothelial cells, L-selectin in lymphocytes, and P-selectin in platelets (224). P-selectin is found both in cytoplasmic granules of platelets, in the Weibel-Palade bodies of endothelial cells, and, after cell activation, on the surface of these cell types (223). P- and E-selectin recognize similar ligands binding various cells of the immune system (223). Thus, P-selectin is a protein expressed by both thrombocytes, which play a key role in homeostasis, and endothelial cells of the vasculature, which are involved in both atherosclerosis and thrombosis (225, 226).
Soluble P-selectin (sP-selectin) can be measured in plasma, and levels correlate with platelet count in healthy individuals (227). In male individuals, smokers, and in individuals with inflammatory disorders, type 2 diabetes, hypertension, and acute coronary syndrome, sP-selectin is elevated, and levels are also associated with increased risk of cardiovascular events (225, 227-233). Furthermore, P-selectin could be a marker for plaque activity and rupture risk (213). In animal studies, P-selectin-targeted molecules may localize atherosclerotic and thrombotic vasculature (213). sP-selectin is reduced by smoking cessation, lipophilic statins, and some BP-lowering medications (227, 234).

In summary, P-selectin may provide insight into the associations between thrombocytes, inflammation, and CVD, and be investigated as a potential biomarker for cardiovascular risk.

Licorice

Licorice is the common name of the extract from the *Glycyrrhiza* species' root, which has been used as a flavoring and herbal medicine since before 4000 years BC (235, 236), Figure 6. Many health benefits have been suggested, in particular in gastrointestinal illnesses such as peptic ulcers, but also infectious diseases, cancer, and CVD (235).

Despite this, ingestion is also known to increase BP, primarily due to the effects of glycyrrhizic acid (GA, also glycyrrhizinic acid, glycyrrhizin) (235). As one of over 300 compounds, GA constitutes 3-10% of licorice and is metabolized into glycyrrhetinic acid when ingested (235, 237). The bioavailability of GA is higher when consumed in its isolated form, suggesting interactions with other constituents of licorice (235). However, the full metabolism of GA, and the effects of the numerous other compounds of licorice, are not entirely known (235).
In the kidney, glycyrrhetinic acid binds to mineralocorticoid receptors, but the affinity is low (235). However, glycyrrhetinic acid also inhibits 11 ß-hydroxysteroid dehydrogenase type 2, which converts cortisol to cortisone (235, 238). This inhibition thus increases the levels of cortisol, which binds more strongly to the mineralocorticoid receptors than cortisone, and causes pseudohyperaldosteronism with hypokalemia, hypernatremia, water retention, and increased BP (235, 238). Animal studies have indicated other potential mechanisms of BP elevation, including direct effects of glycyrrhetinic acid on the liver which increases aldosterone levels, and cerebral elevation of BP when injected locally, without affecting renal resorption of sodium or water (235).

Both the European Union and the WHO have indicated that a daily intake of up to 100 mg of GA is probably safe for most people (239, 240). The Swedish Food Agency has suggested that licorice consumers have a mean daily intake of between 5 and 50 mg of GA, but that the 5% with the highest daily intake consume 100 mg or more (241).

The GA content of licorice products varies greatly. A British study found between 0.29 and 112 mg GA per gram in a variety of products, and a more recent Danish study found that solid licorice products contained between 0.41 and 23.1 mg GA per gram (236, 242). The content of GA depends on commonly known factors including country of origin, but also factors that are more seldomly declared by producers such as plant age and storage conditions (236). Furthermore, although there are around 30 Glycyrrhiza species (e.g., G. glabra or “European licorice”, and G. lepidota or “American licorice”) with a heterogenous composition of more than 400 secondary metabolites and a varied GA content, the species of commercial products are often misclassified (243).

Of 13 previously published studies that have analyzed the effects of whole licorice on BP, referencing 8 study protocols, none have been both randomized and controlled, and reported
independent GA quantifications of the licorice administered (238, 244-253). Furthermore, no previous study has measured home BP.

Finally, ammonium chloride, which does not increase BP, may be used as a flavoring in confectionaries, as an alternative or in addition to licorice (254, 255). It achieves a similar taste, although notably different, and is typically sold as “salty licorice”, even though, as mentioned, it does not always contain any licorice.

**Figure 6** (next page). European licorice, *Glycyrrhiza glabra.*

Drawing from *Flora von Deutschland, Österreich und der Schweiz*, Prof. Dr. Otto Wilhelm Thomé, 1885, Gera, Germany. Reproduced under the CC Public domain.
Markers of Cardiovascular Disease

**Pulse Wave Velocity**

The arterial stiffness described previously can be measured in various ways. One of these is pulse wave velocity (PWV), which is a surrogate marker of arterial stiffness used since the 1920s, expressed in meters per second (26, 28, 256). Pulse wave velocity can be measured invasively using pressure catheters or approximated non-invasively by obtaining the transit time of pulse waves between 2 arterial segments (256, 257). This is preferably made by measuring at 2 sites simultaneously, but alternatively by measuring sequentially with measurements synchronized using an electrocardiogram (256). The PWV is then calculated as the distance between the 2 measurement sites, divided by the travel time between the same sites (257, 258).

Carotid-femoral PWV is considered the gold standard for such non-invasive measurements (2). The disadvantage of this method is that it does not include the most proximal part of the aorta, which represents most of its buffering function (257). Pulse wave velocity has an additive value to traditional risk markers and is one of several screening methods for hypertension-mediated organ damage, which is recommended in the 2023 European Society of Hypertension guidelines (2). Aerobic exercise, especially with high intensity and in individuals with a PWV above 8 m/s, may improve PWV (59).

**Coronary Artery Calcium Score**

The coronary arteries supply the myocardium with oxygen, and insufficient blood flow caused by atherosclerosis, or an imbalance between supply and demand, can result in myocardial infarction (61). The degree of coronary artery calcifications is a marker of atherosclerosis (259), and can be detected using computed
tomography of the heart. These calcifications are usually quantified as the coronary artery calcium score (CACS), and used as a risk marker for CVD, with additive predictive value beyond that of traditional risk markers (260). The usefulness of CACS to guide preventive treatment, however, is debated (261).

**Carotid Artery Plaques**

The brain is supplied with blood via the carotid and vertebral arteries, which are both paired. Atherosclerosis may manifest as the formation of plaques in any of these 4 arteries, and cause ischemic stroke, either by rupture and embolization to more distant vessels, or by occlusion of the artery itself (261, 262). The presence of carotid artery plaques is also associated with cognitive decline and dementia, as well as coronary artery disease (261, 263). Ultrasound is one of many imaging techniques that can be used to evaluate the presence and characteristics of such plaques, thanks to the accessible location of the carotid arteries on the lateral aspects of the neck (263).

**Brain Natriuretic Peptides**

Natriuretic peptides are proteins that are primarily released from the heart, and have hormonal effects on several target organs (264). Brain natriuretic peptide is synthesized in response to increased preload and pressure overload, and increases relaxation of the myocardium, as well as vasorelaxation, and counteracts the effects of RAAS resulting in increased diuresis and decreased sodium retention (244, 264). Its inactive byproduct N-terminal pro-hormone brain natriuretic peptide (NT-proBNP), which has a longer half-life, can be measured in plasma and is primarily used in the clinical setting to rule out heart failure, but may also be elevated in acute coronary syndrome without heart failure, and in pulmonary disease including pulmonary embolism (264). Levels of NT-proBNP are also higher in women and individuals with
Introduction

reduced kidney function, and levels increase with age (264). In indi-
viduals with heart failure, acute coronary syndrome, and pulmo-
nary embolism, elevated levels of NT-proBNP correlate with nega-
tive outcomes (264).

Overarching Aim

The aim of this thesis was to explore home BP and its association
with risk factors and markers of CVD, and to explore the effects of
licorice ingestion on home BP.

Specific Aims

I. The aim of Study I was to cross-sectionally explore the dif-
ference between office and home BP in relation to HbA1c
as well as glycemic status.

II. The aim of Study II was to cross-sectionally characterize
MH and explore its relation to manifestations of vascular
disease, including the extent of CACS and carotid artery
plaques.

III. The aim of Study III was to cross-sectionally explore the
association between sP-selectin and home BP as well as
markers of CVD.

IV. In Study IV, the primary aim was to assess the effects of a
daily licorice intake containing 100 mg of GA on home BP
in healthy volunteers. The secondary aims were to assess
the effects of the same licorice intake on plasma renin, se-
rum aldosterone, and plasma NT-proBNP levels. The ex-
ploratory outcomes were the duration of licorice intake un-
til a change in BP; the duration of subsequent no licorice
intake to normalized BP; and the effects of licorice intake
on body weight as well as plasma sodium and plasma po-
tassium levels.
Methods

Overall Methodology

Studies I-III were cross-sectional and used data from the prospective observational Swedish CArdioPulmonary bioImage Study (SCAPIS). Study IV was a randomized controlled 2x2 cross-over study.

Participants

Studies I-III

In SCAPIS, for a subsample of 5057 participants in Linköping, measurements included home BP as well as additional markers of CVD such as PWV, and markers of inflammation, including hsCRP and sP-selectin. Of these, 5029 participated in the home BP measurements and were considered for inclusion in all 3 studies, Figure 7.

In Study I, all 5029 participants except 4 for whom the hemoglobin levels were below 90 g/L, which was assessed to invalidate the HbA1c measurements, were included.

In Study II, all 4122 participants that did not use BP-lowering medications were included.

In Study III, participants with an hsCRP level below 5 mg/L and a sP-selectin level below 4 standard deviations above mean were included, resulting in a cohort sample of 4548 individuals.
**Table 4. Summary of studies I-IV.**

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Design</strong></td>
<td>Cross-sectional analysis of a prospective, observational study</td>
<td></td>
<td></td>
<td>Randomized, controlled 2x2 crossover study</td>
</tr>
<tr>
<td><strong>Year</strong></td>
<td>2013 to 2018</td>
<td></td>
<td></td>
<td>2023</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>5057 randomly sampled men and women aged 50-64 years</td>
<td></td>
<td></td>
<td>28 healthy adults aged 18-30 years</td>
</tr>
<tr>
<td><strong>Independent variable</strong></td>
<td>Glycemic status and HbA1c</td>
<td>Masked hypertension vs other BP classifications</td>
<td>sP-selectin and hsCRP</td>
<td>Licorice vs control product (salty “licorice”)</td>
</tr>
<tr>
<td><strong>Dependent variable</strong></td>
<td>BP classification and the systolic office-home BP difference</td>
<td>PWV, CACS, carotid artery plaques</td>
<td>BP classification, CACS, carotid artery plaques</td>
<td>Home BP, plasma renin, serum aldosterone</td>
</tr>
<tr>
<td><strong>Statistics</strong></td>
<td>1-way ANOVA test, Jonckheere-Terpstra test for trend, Cochran-Armitage test for trend, logistic regression, linear regression</td>
<td>Mann-Whitney U test, Chi-squared test, linear regression, logistic regression, matching analysis</td>
<td>Kruskal-Wallis test with pairwise comparisons, linear regression</td>
<td>Paired Wilcoxon signed-rank test.</td>
</tr>
</tbody>
</table>

Abbreviations: BP, blood pressure; CACS, coronary artery calcium score; HbA1c, glycated hemoglobin; hsCRP, high-sensitivity C-reactive protein; PWV, pulse wave velocity.
Figure 7. Flow chart of studies I-III.

Participants in SCAPIS Linköping, N = 5057

Did not participate in home BP measurements, n = 28 (0.6%)

Evaluated with home BP, n = 5029 (99.4%)

Study I

Hemoglobin <90 g/L, n = 4 (0.1%)

Included in the study, n = 5025 (99.4%)

Study II

Use of BP-lowering medications, n = 907 (17.9%)

Included in the study, n = 4122 (81.5%)

Study III

hsCRP ≥5 mg/L or sP-selectin ≥4 SD above mean, n = 481 (9.5%)

Included in the study, n = 4548 (89.9%)

Percentages are of the total 5057.
Abbreviations: BP, blood pressure; hsCRP, high-sensitivity C-reactive protein; SCAPIS, The Swedish CArdioPulmonary bioImage Study; SD, standard deviation; sP-selectin, soluble P-selectin.
Study IV

In the Licorice and home blood pressure study, 28 healthy volunteers aged 18 to 30 years were recruited through advertisements in public spaces and on social media in Östergötland County, Sweden, during the first half of 2023. Exclusion criteria were diseases of the cardiovascular, renal, hepatic, or endocrine systems, as well as headache disease, eating disorder, substance use disorder, hormonal treatment, or known intolerance to peanuts or licorice.

Design

Studies I-III

The Swedish CArdioPulmonary bioImage Study is a national prospective observational study of a general population sample aged 50-64 years in Sweden. It is multicenter, with sub studies in Stockholm, Gothenburg, Malmö, Uppsala, Linköping, and Umeå. The baseline examinations were performed between 2013 and 2018 (265), and a second follow-up commenced in January 2024 (266). The plan is for hard end-point data to be available in 2025 (266).

Study IV

Study IV was a non-blinded cross-over study, and drawing ballots were used to randomize participants into two groups. In the intervention then control group (I-C), a 1-week run-in period was followed by a 2-week intervention period, a 2-week washout period, a 2-week control period and a 2-week washout period. In the control then intervention group (C-I), the periods were the same except that the intervention and control period were in the opposite order.

Intervention was pastilles made from Glycyrrhiza glabra from Nature Med S.r.l. (Cosenza, Italy), with a content of 2% GA according to the manufacturer’s labeling, Figure 8A. These were
Methods

sent for independent analysis by Neotron SpL (Modena, Italy) using high-performance liquid chromatography with photodiode-array detection, which showed a true GA content of 2.99 ±0.2 percent by weight.

Control was salty licorice confectionaries from Troll-Gott Konfektyr AB (Årjäng, Sweden), without any GA content according to the manufacturer, Figure 8B. This was confirmed by the same analysis as described above with results showing a GA content below the lower limit of detection (<0.002 percent by weight).

Participants were asked to consume an average of 3.3 grams of the intervention product daily, thus containing a total of 100 mg of GA, during the intervention period, and 2.9 grams of the control product daily during the control period. During the wash-out periods, no licorice was to be consumed.

Figure 8. The licorice used as intervention (A) and the salty licorice used as control (B).

Photo by Peder af Geijerstam.
Measurements

Table 5. An overview of measurements in studies I-IV.

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Office BP</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Home BP</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Body weight</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Body height</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Capillary glucose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Blood HbA1c</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Blood hemoglobin</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood lipid profile</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Plasma creatinine</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Plasma sodium</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Plasma potassium</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Plasma hsCRP</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma sP-selectin</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Plasma NT-proBNP</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Plasma renin</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Serum aldosterone</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Pulse wave velocity</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>CACS</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Carotid artery plaques</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BP, blood pressure; CACS, coronary artery calcium score; HbA1c, glycated hemoglobin; hsCRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro B-type natriuretic peptide; SCAPIS, Swedish CArdioPulmonary bioImage Study; sP-selectin, soluble P-selectin.

Blood Pressure Measurements

Both office and home BP were measured using the validated, semi-automatic, oscillometric Omron M10-IT device (Omron, Kyoto, Kyoto Prefecture, Japan) (267). Measurements were made over the
Methods

brachial artery with the cuff at the level of the heart, and the guidelines of the European Society of Hypertension were followed (123, 268). Before measurements, participants were asked to rest for 5 minutes and abstain from coffee, smoking, and strenuous activity for at least 1 hour.

Office BP was measured in each arm. The arm with the highest mean BP was designated as the reference arm. In studies I-III, measurements were made in the supine position, twice consecutively with 1 min between measurements, and in Study IV, measurements were made once in the seated position.

Home BP was measured in the seated position, 3 times with 1 minute between measurements in the morning and in the evening, respectively. In studies I-III, measurements were made for a total of 7 days, but for the first day only the evening BP was recorded. Thus, the mean morning and evening BP was calculated from 7 and 6 measurements respectively, and the systolic and diastolic home BP was calculated as the mean of these. In Study IV, measurements were made for 3 days during the run-in period, and daily during the intervention, control, and wash-out periods. The mean was first calculated for each evening and morning, and then for each day.

In studies I-III, an average office BP at or above 140 mmHg systolic and/or 90 mmHg diastolic was labelled as hypertensive office BP, and an average office BP below these limits was labelled as normotensive office BP. An average home BP at or above 135 mmHg systolic and/or 85 mmHg diastolic was labelled as hypertensive home BP, and an average home BP below these limits was labelled as normotensive home BP. Based on this categorization of office and home BP, BP was classified as sustained normotension if both were normotensive, sustained hypertension if both were hypertensive, WCH if office BP was hypertensive and home BP was normotensive, and MH if office BP was normotensive and home BP was hypertensive, Table 2.
Blood Samples

In all studies, blood samples were analyzed at the Department of Clinical Chemistry (Linköping University Hospital, Linköping, Sweden), which is accredited according to SS-EN ISO/IEC 17025:2018, except for sP-selectin which was analyzed at SciLifeLab (Clinical biomarkers facility, Uppsala, Sweden).

Markers of Cardiovascular Disease

Carotid-femoral PWV was measured by biomedical scientists using the SphygmoCor® XCEL system (AtCor Medical, Sydney, NSW, Australia), as previously described in detail (269). Before measurements, participants were asked to avoid alcohol, nicotine, caffeine, and heavy meals. Blood pressure cuffs were placed on the left upper arm and right thigh, and the carotid-femoral distance was calculated as the carotid-cuff distance after subtracting the femoral-cuff distance, multiplied by 0.8 in accordance with international guidelines (258). The PWV was derived from simultaneous registrations of the carotid artery signal using an applanation tonometer and the femoral artery signal using the thigh cuff. The procedure was repeated twice, but an additional third measurement was added if the difference between the initial measurements exceeded 0.5 m/s. Participants with 2 values were included, using the mean of these 2 values (269).

Computed tomography was performed using the SOMATOM Definition Flash computed tomography scanner (Siemens Medical Solution, Forchheim, Germany), with the integrated Stellar Detector, and imaging of coronary artery calcium was obtained with non-contrast CT imaging at 100-120 kV with electrocardiogram gating (260). Prior to the assessment, metoprolol was administered to control heart rate, and sublingual glycercyl nitrate was given to dilate the coronary arteries (260). With the exception of participants with the presence of coronary stents or coronary artery bypass grafts, images were reconstructed, and coronary artery
Methods

calcium was scored, as previously described, and expressed as the coronary artery calcium scores (CACS) in Agatston units (260, 270). In the present studies, CACS was dichotomized to <100 and ≥100 because of its skewed distribution and to harmonize with previous presentations in already published SCAPIS studies. In previous studies, an Agatston score of 100 has been associated with coronary heart disease and thus clinical relevance (270).

Carotid artery plaques were assessed bilaterally using a Siemens Acuson S2000 ultrasound scanner with a 9LF linear transducer (Siemens Healthineers, Erlangen, Germany), as previously described in detail (271). Plaques were identified using the Mannheim consensus in both the common carotid artery, the bulb, and the internal carotid artery, and these were summarized into a total plaque number (271). In the present studies, the presence of carotid artery plaques was analyzed as a dichotomized variable of 0 or ≥1 plaque.

Statistical Analyses

Analyses were made using IBM SPSS Statistics (IBM Corp., Armonk, New York, United States) versions 26 to 29, R (R Core Team, Vienna, Austria) versions 4.1.2 to 4.3.3 and RStudio (Posit Software, Boston, MA, USA) versions 2021.09.1 to 2023.12.1+402.

In all studies, distributions were determined using the Kolmogorov-Smirnov test as well as visual assessments. Continuous variables with normal distribution were shown as the mean and standard deviation, and continuous variables with skewed distribution were shown as the median and interquartile range. The exception was BP values, which by convention were presented as normally distributed in all studies except in Study III where the reviewer requested it to be presented as the median and interquartile range. All comparisons were 2-tailed and a significance level of \( P < .05 \) was used. Confidence intervals (CI) were of 95%. For
analyses using adjustments, those were crude, adjusted for age and sex (Model 1), and multiple adjusted (as specified for each study).

**Study 1**

Glycemic status was categorized as diabetes if the participant indicated known diabetes in the questionnaire, if the fasting capillary glucose was at or above 7 mmol/L, or if the HbA1c was at or above 48 mmol/mol; as prediabetes if the participant did not fulfill the criteria for diabetes and if the fasting capillary glucose was at or above 6.1 mmol/L or the HbA1c was at or above 42 mmol/mol; and normoglycemia if none of the above was true. However, participants without known diabetes, with missing fasting capillary glucose and with HbA1c below 42 mmol/mol were not categorized because of the low sensitivity of HbA1c.

Differences in trend were tested using the 1-way ANOVA test for continuous variables with normal distribution, the Jonckheere–Terpstra test for trend for continuous variables with skewed distribution, and the Cochran–Armitage test for trend for categorical variables.

At the advice of a reviewer, a subgroup analysis using logistic regression was made for participants without current BP-lowering medications, comparing associations with glycemic status for participants with WCH vs sustained hypertension and for participants with MH vs sustained normotension. Multiple adjustments included age, sex, smoking status, lipid-lowering medication, waist circumference, estimated glomerular filtration rate, hemoglobin, low-density/high-density lipoprotein ratio and CACS ≥100 (Model 3) as well as PWV in addition to the variables in Model 3 (Model 4).

The office–home BP difference (calculated for each individual by subtracting the systolic home BP from the systolic office BP) was analyzed in relation to HbA1c using linear regression. Adjustments were the same as the above with the addition of diabetes
medication and BP-lowering medication in models 3 and 4. Additionally, a sensitivity analysis was made for participants without BP-lowering medication by request of the reviewer.

**Study II**

In the baseline table, differences between MH and the other BP classifications were tested using a 2-sided Mann-Whitney U test for continuous variables, and the Chi-squared test for categorical variables.

Manifestations of CVD were presented for MH in relation to each of the other 3 BP classifications. Continuous variables (PWV) were shown as the means and 95% CI, and dichotomous variables (CACS $\geq 100$ and carotid artery plaques $\geq 1$) were shown as the odds ratios (OR) and 95% CI. Differences were tested using a linear regression for continuous variables, and a logistic regression using a binomial generalized linear model for categorical variables. Multiple adjustments included age, sex, fasting capillary glucose, and BMI (Model 3). When testing differences between participants with MH and sustained normotension, systolic office BP was also included in Model 3, as we wanted to test whether such an association was also true if the office BP values of the 2 groups were the same.

Ad hoc, by request of the reviewers, a sensitivity analysis of CACS $<400$ vs $\geq 400$ was performed. Also by request of the reviewers, and motivated by the differences in the systolic office BP between participants with MH and sustained normotension, a matching analysis was added for participants with MH and sustained normotension, using a 1 to 3 ratio and propensity score matching for age, sex, and systolic office BP.
Study III

The median and interquartile ranges of sP-selectin, hsCRP, PWV and CACS as continuous variables, and the number and percentages of CACS ≥100 and carotid artery plaques ≥1 as categorical variables, were presented for each of the hypertension phenotypes and sustained normotension, and differences between each of the hypertension phenotypes and sustained normotension were tested using a Kruskal-Wallis test with pairwise comparisons using Conover's many-to-one test. To correct for multiple comparisons, the Holm-Bonferroni method was used.

High-sensitivity C-reactive protein and sP-selectin were then both categorized as quartiles and dichotomized as quartile 4 and quartiles 1-3. Logistic regression using a binomial generalized linear model was used to analyze the association between quartile 4 vs quartiles 1-3 of sP-selectin and hsCRP, respectively, and each of the hypertension phenotypes vs sustained normotension, and presented as the OR and 95% CI. Multiple adjustments included age, sex, smoking status, diagnosis of diabetes, diagnosis of hyperlipidemia, hsCRP and sP-selectin (Model 3). The same analysis was used to evaluate the association between quartile 4 vs 1-3 of sP-selectin and hsCRP, respectively, and markers of CVD, but with the addition of diagnosis of hypertension to the adjustments in Model 3.

Finally, linear regression was used to analyze the associations between sP-selectin and hsCRP as continuous variables and each of the BP classification and presented as the estimates and 95% CI as well as 2 figures with the proportion of each BP classification on the Y-axis vs the level of sP-selectin and hsCRP, respectively, on the X-axis.
Study IV

Results of blood samples that were left censored were converted as the highest possible value divided by the square root of 2, which is one of many substitution methods and in this instance conservative (assuming a censored distribution which is skewed towards the non-censored values) (272). This was performed for plasma renin levels below the lower limit of quantification (<1.7 mIU/L) which affected 2 of the 84 (2.4%) samples analyzed; for serum aldosterone levels below the lower limit of quantification (<50 µmol/L) which affected 3 of the 84 (3.6%) samples analyzed; and for NT-proBNP levels below the lower limit of detection (<10 ng/L) which affected 2 of the 21 (9.5%) samples analyzed for the quartile of subjects with the most pronounced renin and aldosterone suppression.

All continuous variables were determined to be skewed by visual assessment, and thus presented as the median and interquartile range, except for BP measurements which by convention were presented as the mean and standard deviation.

The change in systolic and diastolic BP was presented as the mean and 95% CI difference between the first and last 3 days of the intervention and control periods, respectively. The change in all other measurements was presented as the mean and 95% CI percentage change between the run-in period and the end of the intervention and control period, respectively. For all measurements, the difference between the change during the intervention and control period was tested using a paired Wilcoxon signed-rank test.

To analyze the number of days until the BP had changed, and whether a plateau phase was reached or not, the systolic and diastolic BP during each of days 1 to 14 of the intervention period was compared with the 3 days preceding the intervention period and presented as the absolute mean and 95% CI of each day, as well as the mean change and 95% CI vs the 3 days preceding the
intervention period, and this difference was tested using a paired Wilcoxon signed-rank test. To analyze the number of days during the wash-out period until the BP was no longer different from the 3 days preceding the intervention period, a similar table was presented with days 1 to 14 of the post-intervention washout period.

Ad hoc, the quartile of participants with the largest relative change in renin and aldosterone during the intervention period, calculated as the ratio of the renin at the end of the intervention to that at the end of the control period plus the same ratio for aldosterone, was analyzed as above.

**Study IV Follow-Up (Ad-Hoc)**

To study the effects of even lower doses of licorice in the quartile of participants with the most pronounced suppression of the plasma renin and serum aldosterone values, a follow-up prospective observational study was planned and performed ad hoc, after the completion of Study IV. This quartile (n = 7) of participants was invited to participate during the second half of 2023.

After a run-in period of 3 days, participants were asked to consume licorice corresponding to 20 mg of GA per day for 2 weeks, directly followed by another 2 weeks of a daily licorice intake corresponding to 50 mg of GA. During the entire study, home BP was measured daily using the same methods and instructions as in Study IV, and at the end of each period (thus, on a total of 3 occasions), body weight was measured and blood samples (plasma sodium, plasma potassium, plasma creatinine, plasma NT-proBNP, plasma renin, and serum aldosterone) were collected.

The mean and 95% CI of all measurements during the run-in and at the end of Period 1 and Period 2 were calculated, as well as the mean and 95% CI change between the run-in period and Period 1 (delta period 1) and the run-in period and Period 2 (delta period
2). Delta values with confidence intervals that did not contain zero were considered statistically significant.

Ethics

All studies were approved by the Swedish Ethical Review Authority (Dnr 2010-228-31M and Dnr 2018/478-31 for studies I-III, Dnr 2022-06163-01 for Study IV, and Dnr 2023-04688-02 for Study IV Follow-Up) and adhered to the Declaration of Helsinki.
Results

Study I

Of 5025 included participants, 907 (18.0%) had BP-lowering medication, and 370 (7.4%) had diabetes, Table 6. All hypertension phenotypes increased in prevalence with glycemic status, $P$ for trend = .006 for WCH and $P$ for trend <.001 for masked and sustained hypertension, Figure 9.

CACS $\geq 100$ was present in 390 (9.8%) of those with normoglycemia, 102 (15.1%) of those with prediabetes, and 100 (27.0%) of those with diabetes, $P$ for trend <.001. The systolic office-home BP difference decreased with increased HbA1c, $P = .012$ in Model 3, but not after additional adjustment with PWV, $P = .291$.

In participants without BP-lowering medication, MH vs sustained normotension increased with dysglycemia, $P = .036$ in Model 3, but not after also adjusting for PWV. In these participants, additionally, the systolic office-home BP difference was no longer associated with the HbA1c value, $P = .793$.

Table 6 (next page). Baseline variables according to glycemic status (200).

All values are the numbers and percentages and median and interquartile range, for categorical and continuous variables, respectively, except for blood pressure variables which are the means and standard deviations. Reproduced with minor adjustments by permission of Oxford University Press from af Geijerstam et al. Home Blood Pressure Compared With Office Blood Pressure in Relation to Dysglycemia. *American Journal of Hypertension*, 35(9):810-819, September 2022.

Abbreviations: BP, blood pressure; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
<table>
<thead>
<tr>
<th></th>
<th>Normoglycemia, n = 3979</th>
<th>Prediabetes, n = 676</th>
<th>Diabetes, n = 370</th>
<th>Total, N = 5025</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 (53-61)</td>
<td>58 (55-62)</td>
<td>60 (56-63)</td>
<td>57 (54-61)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Ever-smokers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous</td>
<td>1200 (30.2)</td>
<td>241 (35.7)</td>
<td>136 (36.8)</td>
<td>1577 (31.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Current</td>
<td>343 (8.6)</td>
<td>81 (12.0)</td>
<td>46 (12.4)</td>
<td>470 (9.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>26 (24-28)</td>
<td>28 (25-31)</td>
<td>30 (27-33)</td>
<td>26 (24-29)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>91 (82-99)</td>
<td>98 (89-105)</td>
<td>104 (97-113)</td>
<td>92 (84-101)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.4 (5.2-5.7)</td>
<td>6.2 (5.9-6.5)</td>
<td>7.7 (6.8-9.4)</td>
<td>5.6 (5.2-5.9)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Glycated hemoglobin (mmol/mol)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35 (33-37)</td>
<td>37 (35-40)</td>
<td>48 (42-58)</td>
<td>35 (33-38)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Estimated GFR (mL/min/1.73m²)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>82 (74-92)</td>
<td>84 (74-93)</td>
<td>88 (77-96)</td>
<td>82 (74-92)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>5.5 (4.9-6.2)</td>
<td>5.3 (4.6-6.0)</td>
<td>4.6 (3.7-5.4)</td>
<td>5.4 (4.8-6.1)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Low-density lipoprotein (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.3 (2.7-3.9)</td>
<td>3.1 (2.5-3.7)</td>
<td>2.4 (1.8-3.2)</td>
<td>3.2 (2.6-3.9)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>High-density lipoprotein (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6 (1.3-2.0)</td>
<td>1.5 (1.2-1.8)</td>
<td>1.3 (1.0-1.6)</td>
<td>1.6 (1.3-1.9)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 (0.8-1.4)</td>
<td>1.1 (0.8-1.5)</td>
<td>1.3 (1.0-2.1)</td>
<td>1.0 (0.8-1.5)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>LDL/HDL ratio</strong></td>
<td>2.0 (1.5-2.7)</td>
<td>2.1 (1.5-2.7)</td>
<td>1.9 (1.3-2.6)</td>
<td>2.0 (1.5-2.7)</td>
<td>.022</td>
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<tr>
<td><strong>Total coronary artery calcium score ≥100</strong></td>
<td></td>
<td></td>
<td></td>
<td>592 (11.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Pulse wave velocity (m/s)</strong></td>
<td></td>
<td></td>
<td></td>
<td>87 (7.9-9.7)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Current medication</strong></td>
<td>Hypertension</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>564 (14.2)</td>
<td>171 (25.3)</td>
<td>172 (46.5)</td>
<td>907 (18.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Hyperlipidemia</td>
<td></td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>166 (4.2)</td>
<td>76 (11.2)</td>
<td>121 (32.7)</td>
<td>363 (7.2)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>181 (48.9)</td>
<td>181 (3.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Office BP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Systolic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>131 (17)</td>
<td>137 (18)</td>
<td>139 (17)</td>
<td>133 (18)</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>83 (10)</td>
<td>85 (11)</td>
<td>85 (10)</td>
<td>83 (10)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Home BP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Systolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>119 (14)</td>
<td>124 (14)</td>
<td>129 (13)</td>
<td>121 (14)</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>78 (9)</td>
<td>80 (9)</td>
<td>81 (8)</td>
<td>78 (9)</td>
<td>.002</td>
</tr>
<tr>
<td><strong>Systolic office-home BP difference (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td>11.9 (11.6)</td>
<td>.282</td>
</tr>
</tbody>
</table>
Table 7. Blood pressure classifications, and subtypes according to glycemic status (200).

<table>
<thead>
<tr>
<th></th>
<th>Total, N = 5025</th>
<th>Normoglycemia, n = 3979</th>
<th>Prediabetes, n = 676</th>
<th>Diabetes, n = 370</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Office blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Normotensive</td>
<td>3187 (63.4)</td>
<td>2645 (66.5)</td>
<td>358 (53.0)</td>
<td>184 (49.7)</td>
<td></td>
</tr>
<tr>
<td>Hypertensive</td>
<td>1838 (36.6)</td>
<td>1334 (33.5)</td>
<td>318 (47.0)</td>
<td>186 (50.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Home blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Normotensive</td>
<td>3824 (76.1)</td>
<td>3144 (79.0)</td>
<td>459 (67.9)</td>
<td>221 (59.7)</td>
<td></td>
</tr>
<tr>
<td>Hypertensive</td>
<td>1201 (23.9)</td>
<td>835 (21.0)</td>
<td>217 (32.1)</td>
<td>149 (40.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Blood pressure classifications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained normotension</td>
<td>2933 (58.4)</td>
<td>2473 (62.2)</td>
<td>314 (46.4)</td>
<td>146 (39.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sustained hypertension</td>
<td>947 (18.8)</td>
<td>663 (16.7)</td>
<td>173 (25.6)</td>
<td>111 (30.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>White coat hypertension</td>
<td>891 (17.7)</td>
<td>671 (16.9)</td>
<td>145 (21.4)</td>
<td>75 (20.3)</td>
<td>.006</td>
</tr>
<tr>
<td>Masked hypertension</td>
<td>254 (5.1)</td>
<td>172 (4.3)</td>
<td>44 (6.5)</td>
<td>38 (10.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Hypertension subtypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined hypertension</td>
<td>1261 (25.1)</td>
<td>914 (23.0)</td>
<td>225 (33.3)</td>
<td>122 (33.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diastolic hypertension</td>
<td>353 (7.0)</td>
<td>260 (6.5)</td>
<td>56 (8.3)</td>
<td>37 (10.0)</td>
<td>.004</td>
</tr>
<tr>
<td>Systolic hypertension</td>
<td>478 (9.5)</td>
<td>332 (8.3)</td>
<td>81 (12.0)</td>
<td>65 (17.6)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Figure 9. Proportional stacked bar plot of blood pressure classification in relation to glycemic status in all participants (200).

Results

Study II

Of 4122 included participants, 172 (4.2%) had MH and 376 (9.1%) had CACS ≥100. Compared with participants with sustained normotension, those with MH were more often men (66.9 vs 46.2%) and had a higher BMI (mean 28.0 vs 25.6 kg/m²), higher fasting glucose value (mean 5.0 vs 5.5 mmol/L), lower high-density lipoprotein fraction (mean 1.5 vs 1.7 mmol/L), and higher hsCRP value (mean 2.7 vs 1.6 mg/L), all \( P < .001 \). Furthermore, participants with MH vs sustained normotension had higher PWV (mean 9.3 vs 8.3 m/s, \( P < .001 \)), and more often CACS ≥100, OR 1.65 (95% CI 1.02-2.68), Table 8 and Figure 10. In the sensitivity analysis, participants with MH vs sustained normotension also more often had CACS ≥400, OR 2.73 (95% CI 1.27-5.89), but not after adjusting for age and sex. Those with MH had higher odds of carotid artery plaques in the unadjusted model, OR 1.48 (95% CI 1.08-2.02), but not after adjustments.

Comparing participants with MH to those with sustained hypertension, there was no difference in CACS ≥100 or carotid artery plaques ≥1, but PWV was lower, mean 9.3 (95% CI 9.1-9.6) vs 10.0 (95% CI 9.7-10.2) m/s, \( P < .001 \), Figure 10.
**Figure 10.** A box plot of pulse wave velocity in masked hypertension compared with sustained normotension, white-coat hypertension and sustained hypertension respectively (165).

The boxplot includes the median, the box extending between the 25th to the 75th percentile (the interquartile range, IQR) and its whiskers extending between the IQR times 1.5; the violin plot illustrates the relative distribution of observations; and the left-sided vertical bar plot shows the actual observations. Differences between groups were tested using a 2-sided Mann-Whitney U test.

Abbreviations: m/s, meters per second.

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Table 8. Markers of cardiovascular disease in participants with masked hypertension compared to sustained normotension (165).

<table>
<thead>
<tr>
<th></th>
<th>Crude</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Pulse wave velocity (m/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained normotension</td>
<td>8.3 (8.2-8.3)</td>
<td>8.3 (8.2-8.4)</td>
<td>8.3 (8.2-8.4)</td>
</tr>
<tr>
<td>Masked hypertension</td>
<td>9.4 (9.2-9.6)</td>
<td>9.4 (9.1-9.7)</td>
<td>9.3 (9.1-9.5)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery calcium score ≥100</td>
<td>2.47 (1.60-3.83)</td>
<td>1.88 (1.19-2.97)</td>
<td>1.65 (1.02-2.68)</td>
</tr>
<tr>
<td>Carotid artery plaques ≥1</td>
<td>1.48 (1.08-2.02)</td>
<td>1.33 (0.97-1.83)</td>
<td>1.12 (0.80-1.56)</td>
</tr>
</tbody>
</table>

Differences between continuous variables were tested using linear regression, and differences between categorical variables were tested using logistic regression (using a binomial generalized linear model). For the logistic regression, sustained normotension was the reference (odds ratio = 1).

Abbreviations: CI, confidence interval; m/s, meters per second; OR, odds ratio.

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Study III

Of 4548 included participants, 775 (17.0%) had BP-lowering medication. The sP-selectin value was higher in all hypertension phenotypes vs sustained normotension, median 34.9 for WCH, 35.8 for MH, and 36.8 for sustained hypertension, vs 33.5 ng/mL, \(P = .011\), \(P = .030\), and \(P < .001\), respectively. The hsCRP value was also higher in participants with any of the hypertension phenotypes vs sustained normotension, median 0.9 for WCH, 1.2 for MH, and 1.2 for sustained hypertension, vs 0.8 mg/L, \(P < .001\) for all comparisons, Figure 11.

Participants with a sP-selectin value in quartile 4 vs 1-3 more often had WCH, OR 1.29 (95% CI 1.07-1.54), and sustained hypertension, OR 1.67 (95% CI 1.40-1.98), compared with sustained normotension. These participants also more often had CACS \(\geq 100\), OR 1.26 (95% CI 1.03-1.55) and carotid artery plaques, OR 1.16 (95% CI 1.01-1.33), but not after adjustment for age and sex. Analyzing sP-selectin as a continuous variable, higher values were related to decreased proportion of participants with sustained normotension, estimate -0.27 (95% CI -0.47 to -0.07) and increased proportions of participants with sustained hypertension, estimate 0.28 (95% CI 0.11-0.46), Figure 12.

Participants with an hsCRP value in quartile 4 vs 1-3 more often had WCH, OR 1.37 (95% CI 1.14-1.64), MH, OR 2.31 (95% CI 1.72-3.10), and sustained hypertension, OR 2.25 (95% CI 1.89-2.66), compared with sustained normotension. These participants also more often had carotid artery plaques, OR 1.16 (1.00-1.34) after multiple adjustments, but there was no difference in the odds for CACS \(\geq 100\). Analyzing hsCRP as a continuous variable, higher values were related to decreased proportion of participants with sustained normotension, estimate 5.59 (95% CI -7.83 to -3.34) and
increased proportions of participants with MH and sustained hypertension, estimates 1.10 (95% CI 0.31-1.88) and 4.04 (2.25-5.83), respectively.

**Figure 11.** Box plot of high sensitivity C-reactive protein (hsCRP) values in relation to blood pressure classifications (273).

Pairwise comparisons between sustained normotension and hypertension phenotypes were tested using a Kruskal–Wallis test with pairwise comparisons using Conover’s many-to-one test and the Holm–Bonferroni method to correct for multiple comparisons.

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**Figure 12.** Proportional area plots of each blood pressure classification in relation to sP-selectin (273).

The fit line (black) and 95% CI (shaded) illustrates the estimate of the linear regression. The dark grey areas represent the proportion of the blood pressure classification for each level sP-selectin.

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Study IV

Of 28 participants, all completed the study, Figure 13. The median age was 24.0 (Q1-Q3 22.8-27.0) years, and at run-in, the mean systolic home BP was 106.4 (±9.5) mmHg.

During the intervention period the systolic home BP increased with a mean (95% CI) 3.1 (0.8-5.4) mmHg compared with -0.3 (-1.8 to 1.3) mmHg during the control period, \( P = .018 \), Table 9. The increase in systolic home BP began day 5, mean (95% CI) 1.7 (0.2-3.3) mmHg difference compared with the 3 days preceding the intervention period, \( P = .037 \), Figure 14.

At the end of the intervention vs control period, plasma renin and serum aldosterone levels had decreased by a mean (95% CI) -30.0 (-56.7 to -3.3) vs 15.8 (12.8 to 44.4) %, and -45.1 (-61.5 to -28.7) vs 8.2 (-14.7 to 31.1) %, respectively, Figure 15. Comparing the intervention and the control period, the plasma sodium value increased slightly during the former, \( P = .028 \), but comparing the end of the intervention period to the run-in period, no difference was seen for neither of these periods. For the plasma creatinine and potassium values, no significant differences in change from the run-in period were observed between the intervention and control periods.

During the wash-out period after the intervention, the systolic BP decreased to a level which was numerically but not significantly different from that of the 3 days preceding the intervention period, \( P = .141 \). However, the diastolic BP continued to increase numerically and remained different from the 3 days preceding the intervention period at the end of the wash-out period, \( P = .001 \).

In subgroup analyses of the quartile of participants (n = 7) with the highest relative decrease in renin and aldosterone, the body weight and the plasma NT-proBNP value increased during the intervention vs control period with a mean (95% CI) 1.3 (0.9 to 1.7) vs -1.0 (-1.8 to -0.2) % for body weight, and a mean (95% CI)
204.1 (-11.6 to 419.7) vs 72.4 (-52.2 to 197.1) % for the plasma NT-proBNP value, both $P = .016$. For these 7 participants, the plasma potassium value also decreased during the intervention period when compared to the run-in period, -7.0 (95% CI -12.0 to -2.0) %, and this was numerically different to the change during the control period, -1.5 (-11.9 to 8.9) %, $P = .078$.

**Figure 13.** Flow chart of the study design (31).

**Table 9.** Change in home blood pressure, as well as weight and blood samples, during the intervention vs control periods (31).

<table>
<thead>
<tr>
<th></th>
<th>Mean (95% CI) difference between the first and last 3 days of the intervention period, n = 28</th>
<th>Mean (95% CI) difference between the first and last 3 days of the control period, n = 28</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Home blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>3.1 (0.8 to 5.4)</td>
<td>-0.3 (-1.8 to 1.3)</td>
<td>.018</td>
</tr>
<tr>
<td>Diastolic</td>
<td>1.9 (0.5 to 3.3)</td>
<td>0.6 (-0.8 to 1.9)</td>
<td>.236</td>
</tr>
<tr>
<td><strong>Mean (95% CI) percentage change between the run-in period and the end of the intervention period, n = 28</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>0.5 (-0.2 to 1.2)</td>
<td>-0.4 (-1.0 to 0.2)</td>
<td>.023</td>
</tr>
<tr>
<td>Plasma creatinine</td>
<td>-4.8 (-8.1 to -1.5)</td>
<td>-2.9 (-5.9 to 0.1)</td>
<td>.078</td>
</tr>
<tr>
<td>Plasma potassium</td>
<td>-1.2 (-3.4 to 1.1)</td>
<td>-0.1 (-2.6 to 2.4)</td>
<td>.354</td>
</tr>
<tr>
<td>Plasma sodium</td>
<td>0.2 (-0.3 to 0.6)</td>
<td>-0.3 (-0.6 to 0.1)</td>
<td>.028</td>
</tr>
<tr>
<td>Plasma renin</td>
<td>-30.0 (-56.7 to -3.3)</td>
<td>15.8 (-12.8 to 44.4)</td>
<td>.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum aldosterone</td>
<td>-45.1 (-61.5 to -28.7)</td>
<td>8.2 (-14.7 to 31.1)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aldosterone/renin ratio</td>
<td>25.4 (-7.0 to 57.9)</td>
<td>7.5 (-10.9 to 26.0)</td>
<td>.546</td>
</tr>
<tr>
<td>Plasma NT-proBNP</td>
<td>85.6 (19.7 to 151.5)</td>
<td>25.0 (-12.7 to 62.7)</td>
<td>.033&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Difference between the intervention and control period was tested using a paired Wilcoxon signed-rank test.

a. Bonferroni-corrected significance level for P value for the secondary outcome analyses (renin, aldosterone, and NT-proBNP) = .0167.

Abbreviations: mmHg, millimeters of mercury; NT-proBNP, N-terminal pro B-type natriuretic peptide.

**Figure 14.** Box plot of the systolic home blood pressure during each day of the intervention period compared with the mean of the 3 preceding days (31).

Results are based on measurements from all 28 participants except for day 13, which is based on measurements from 27 (96.4%) of the participants. Difference between each day of the intervention period and the mean of the 3 preceding days (the 3-day run-in period for the I-C group and the last 3 days of the post-control washout period for the C-I group) were tested using a paired Wilcoxon signed-rank test. The boxplot includes the median, the box extending between the 25th and the 75th percentiles (the IQR), and its whiskers extending between the IQR x 1.5; the violin plot illustrates the relative distribution of observations, and the left-sided vertical dot plot shows the actual observations.

**Figure 15.** Box plots of the percentage change in plasma renin and serum aldosterone values from the run-in to the intervention and control periods (31).

The difference between the periods was tested using a paired Wilcoxon signed-rank test. The boxplot includes the median, the box extending between the 25\(^{th}\) and the 75\(^{th}\) percentiles (the IQR), and its whiskers extending between the IQR \(\times 1.5\); the left-sided vertical dot plot shows the actual observations. Bonferroni-corrected significance level for \(P\) value = .0167.

For renin, an observation during the control period with an increase of 226.7\% is not shown.

Study IV Follow-Up (Ad-Hoc)

Of 7 invited individuals, 3 (43%) accepted participation, with inclusion dates between 19th September 2023 and 23rd February 2024. No results were significant due to the low participation rate. The systolic BP increased numerically during the licorice intake periods for 2 of 3 participants, Figure 16A, the diastolic BP decreased numerically during the licorice intake period for 2 of 3 participants, Figure 16B, and both plasma renin and serum aldosterone levels had decreased numerically until the end of the second licorice intake period for all 3 participants, Figure 16C and 16D.

Figure 16 (next page). Results of systolic (A) and diastolic BP (B), as well as plasma renin (C) and serum aldosterone (D), from Study IV Follow-Up. Not previously published.
Discussion

Study I

Dysglycemia was related to increased systolic office-home BP difference. However, the inverse association between HbA1c and the systolic office-home BP difference was no longer significant in the multiple adjusted model which included PWV. The latter is a measurement of arterial stiffness, which is associated with both dysglycemia and the office-home BP difference (274, 275). In fact, both PWV and pulse pressure, which is also a marker of arterial stiffness, are associated with subsequent development of dysglycemia (276-278). For example, in a Swedish study of 2450 individuals evaluated with PWV using an instrument from the same manufacturer as the one used in SCAPIS, the hazard ratio for incident diabetes for those with PWV in tertile 3 vs 1 was 3.24 after multiple adjustments (279). The associations between both PWV and the office-home BP difference, and PWV and dysglycemia, could explain why adding PWV to the model cancelled out the association between dysglycemia and the office-home BP difference. Finally, PWV had a high missing rate and thus reduced the power of the regression analysis (280).

We also found an association between dysglycemia and increased prevalence of MH, which is in line with previous studies (281). In our study, 10% of the participants with diabetes had MH, which is comparable to a 2022 study of 1082 individuals with diabetes, in which 12.7% had MH (282). When comparing this prevalence figure to other studies, however, it is more akin to individuals without diabetes. A 2008 systematic review of several heterogeneous cohorts both in terms of age and co-morbidities found a prevalence of MH which varied between 8 and 17% using ambulatory BP, and 9 and 15% using home BP measurements
(disregarding the studies in which hypertension classification was based on only systolic or diastolic BP) (175). Apart from the 2022 study already mentioned, studies of individuals with diabetes have shown much higher prevalence figures, but they have only evaluated individuals with normotensive office BP, which naturally increases the prevalence figure. For example, in a 2013 study of 9691 individuals, 29.3% vs 18.8% of those with vs without diabetes had MH (281). In 3 smaller studies between 2007-2022, with cohorts of 45, 81, and 150 individuals with diabetes, 47%, 46.9%, and 66%, respectively, had MH (283-285). It is also possible that, since these studies only included individuals with known diabetes, subjects had a more long-standing or severe diabetes than in our study, and thus higher prevalence of MH. Another 2020 study of 4756 individuals with diabetes, using ambulatory BP monitoring, found an MH prevalence of 41.2%, but they did not use the consensus BP cut-off for out-of-office BP of 135/85 mmHg, but rather 130/80 mmHg, which may have increased the MH prevalence (286). In summary, the 10% MH prevalence in individuals with diabetes in our study was lower than in most previous studies, but not unreasonable when considering that the individuals with diabetes in our sample may have had diabetes of shorter duration or lower severity than previous studies.

There are many potential explanations as to why the home vs office BP is relatively higher in individuals with dysglycemia vs normoglycemia. Individuals with dysglycemia and MH have several factors in common, which could explain some covariation e.g., dyslipidemia, obesity, smoking, higher age, and male sex. Previous explanatory suggestions include nocturnal hypertension and that BP-lowering medications could have a more pronounced effect on (or be more diligently used ahead of) office vs out-of-office BP measurements (190, 281, 287). The latter could explain why there was no longer any association between HbA1c and the office-home BP difference when only participants without BP-lowering
medication were evaluated. In individuals with diabetes, MH is also more common when diagnosed using night-time vs daytime out-of-office BP, but such MH may partly be explained by obstructive sleep apnea (174, 190, 288). Pulse wave velocity is also higher in both individuals with hypertension and individuals with dysglycemia and, as discussed in the Introduction section, may be influenced by factors such as insulin secretion (27, 32, 33, 36-38).

Masked hypertension is also more common in individuals with obesity (289). Obesity is associated with hypertension in general, and this could relate to increased activity in the sympathetic nervous system, mediated by insulin and/or leptin (290). Such sympathetic activity may also stimulate RAAS, and adipose tissue itself may contribute to increased angiotensin II (290). Together, such increased sympathetic, insulin and RAAS activity may stimulate reabsorption of sodium in the kidneys, and ultimately higher BP (290). Sympathetic activity, which is also increased in individuals with diabetes, has also been suggested as a possible link between diabetes and MH (174, 291). Furthermore, obstructive sleep apnea, which is likewise linked to obesity, also stimulates the sympathetic nervous system (290).

Individuals with BP-lowering medications and MH (i.e., masked uncontrolled hypertension) have overall higher sympathetic tone, measured by catecholamine levels, and relatively higher sympathetic tone out-of-office vs at the office (291). However, this association remains even after adjustments for BMI and diabetes, indicating that those conditions do not fully explain this relation (291).

Finally, the increased prevalence of CACS ≥100 with dysglycemia is in line with previous studies. In a study of 1552 individuals with and without type 2 diabetes which underwent CT scanning between 1996 and 1999, individuals with diabetes had a higher median CACS (292). In fact, the diagnosis of diabetes was the strongest risk factor after male sex, with a numerically higher OR for
CACS >0 than both age and BMI, and the third strongest risk factor after male sex and age for the extent of CACS (292). The increased CACS in individuals with type 2 diabetes may relate to both environmental and genetic factors (293). In a 2023 meta-analysis of 108 studies including 113,406 individuals, those with CACS >0 were more likely to have diabetes (294). Elevated CACS increases the risk of cardiovascular events, and is therefore a clinically important risk factor (294).

In summary, we found that dysglycemia was associated with a relatively higher home vs office BP, and thus increased prevalence of MH, as well as increased CACS as a marker of CVD. Our results from a large cohort of the general population confirm and strengthen these already observed associations. Explanations for this relation may include increased insulin, RAAS, and sympathetic activity, as well as common factors such as dyslipidemia, obesity, male sex, and arterial stiffness.

Study II

In Study II, we found that individuals with MH vs sustained normotension, consistent with several previous studies, more often were men, and had higher BMI and fasting glucose levels compared with those with sustained normotension (173, 190, 289, 295). In fact, apart from the obvious risk factor of borderline hypertension in office BP, age, sex, and BMI has been suggested factors to be included in risk assessments for potential MH (296). Whether the increased prevalence of MH in men is independent or related to other factors such as the metabolic syndrome, is not known.

We found a prevalence of MH of 4.2%. In individuals without BP-lowering medication, using home BP and the same diagnostic criteria as our, previous studies have consistently found a higher MH prevalence of between 8-29.5%. However, participant
characteristics may partly explain such variations. In a study of a cohort with age, sex, and BMI characteristics rather similar to ours, 8% had MH (171). Other studies found prevalence numbers of between 13.6 to 29.5%, but differed significantly to our in terms of age, sex, and/or obesity prevalence which limits comparability (172-174). In a study of 2004 individuals without known hypertension but referred to a hypertension center and evaluated with ambulatory BP measurements, 5.2% had MH (121), but this lower figure could be influenced by selection bias in the referral process towards individuals with elevated office BP.

We also found that those with MH vs both sustained normotension and WCH had higher mean hsCRP. In a 2011 study of 430 individuals, evaluated using office and ambulatory BP, participants with MH vs sustained normotension also had higher hsCRP. The authors argued that the increased hsCRP could reflect subclinical vascular dysfunction (148). Interestingly, participants with WCH in that study had numerically higher hsCRP than the participants with MH, whilst in our study, the degree of the association was vice versa.

In our study, individuals with MH vs both sustained normotension and WCH also had lower high-density lipoprotein. This has previously been shown in a cohort of 4261 individuals, using office and home BP, but with significant difference only vs WCH, and not sustained normotension (297). Reduced high-density lipoprotein was also one of the first findings on MH, together with elevated plasma insulin levels, in a 1993 study on what was then referred to as “hypertension at home” (167).

Masked hypertension was also associated with arterial stiffness. The PWV was 9.3 vs 8.3 m/s for those with MH vs sustained normotension. Thus, none of the groups had a mean PWV above the threshold for hypertension-mediated organ damage of 10 m/s proposed by the European Society of Hypertension nor the cut-off of 9.6 m/s which would be the cut-off if it was adapted from direct
PWV measurement cut-offs without rounding off (2, 258). However, it has been shown that a 1 m/s increase in PWV is associated with a more than 10% increased risk in cardiovascular events and mortality (59). In the Framingham Heart Study, the risk of a first major CVD event increased for each quartile of PWV, up to a hazard ratio of 3.4 after a mean of 7.8 years when comparing participants in the highest (≥11.8 m/s) vs the lowest quartile (<7.8 m/s) of PWV (298). It is therefore not unreasonable to consider that the difference in our study was clinically significant. Finally, the normal mean PWV of our cohort is in line with the low MH prevalence.

Interestingly, there was no difference in PWV between those with MH and WCH, and the PWV was higher in those with sustained hypertension vs MH, mean 10.0 vs 9.3 m/s. Thus, the mean PWV of those with sustained hypertension met the threshold for hypertension-mediated organ damage (2). Previous studies have found similar results, with individuals with sustained hypertension having a mean PWV of between 8.2 and 11.9 m/s (152). These findings support the notion that measurements of office BP have additional value to those of home BP, and that WCH is not innocuous (152).

Masked hypertension was also associated with higher OR for CACS ≥100 vs sustained normotension. With an absolute risk for CACS ≥100 in the entire cohort of 9.1%, the OR of 1.65 may be regarded as clinically significant, as it implies both increased risk and changes in therapeutic considerations. CACS >100 is associated with an increased risk of CVD and is used by several major guidelines to suggest initiation or consideration of initiation of lipid-lowering therapy and in some guidelines also acetylsalicylic acid (299). The association between MH and CACS has previously been suggested as a trend but not found to be significant (191).

In summary, we found a lower prevalence of MH than in previously published studies, perhaps explained by differences in methodology or cohort characteristics. Masked hypertension was
associated with higher hsCRP, PWV, and CACS, and lower high-density lipoprotein, all of which confirmed and strengthened findings of previous studies. The difference in PWV and CACS compared with sustained normotension was on a scale that may be considered clinically meaningful. Interestingly, the PWV was not different from participants with WCH, confirming previous indications that both these conditions are associated with negative prognostic factors for CVD.

Study III

In Study III, we found that both sP-selectin and hsCRP were associated with sustained hypertension, and that hsCRP was also associated with both MH and WCH.

High-sensitivity CRP was higher in participants with both WCH, MH, and sustained hypertension, compared with sustained normotension. The OR for hsCRP in quartile 4 vs quartiles 1-3 was higher in all these phenotypes even after full adjustments, including sP-selectin. Thus, hsCRP was independently associated with all hypertension phenotypes. The same was true for sP-selectin, except that the OR for WCH was not significant, OR 1.18 (0.98-1.44), after multiple adjustments including hsCRP, and that the OR for MH was not different than for sustained normotension. Higher CRP and hsCRP has previously been seen in individuals with each of the hypertension phenotypes vs sustained normotension when diagnosed using office and ambulatory BP (148-150). However, P-selectin in relation to hypertension phenotypes diagnosed using home BP has not been previously studied. E-selectin has been shown to be higher in individuals with sustained hypertension, but not WCH, vs sustained normotension, again using ambulatory BP measurements (300). However, P-selectin and E-selectin may relate differently with markers of CVD and is thus not directly comparable (301). Although no causality or longitudinal association has been established, the association with P-selectin could be
clinically significant, as P-selectin levels are associated with plaque activity, incident cardiovascular events, and plaque rupture risk (213, 232). Finally, the absence of an association with MH could relate to the higher prevalence of BP-lowering medications in those participants, which may decrease not only BP, but also P-selectin (302), and for which we did not adjust.

Soluble P-selectin, but not hsCRP, was associated with CACS $\geq 100$, but not after adjustments for age and sex. Both sP-selectin and hsCRP were associated with carotid artery plaques $\geq 1$, and the association for hsCRP was independent of all adjusted variables including sP-selectin. A previous study by Bielinski et al of 6025 individuals with a mean age of 64 years showed that with each increased standard deviation (SD) of the P-selectin value, CACS increased with 75 Agatston units, after adjustments (233). The hazard ratio for a coronary artery event was likewise increased with a mean hazard ratio of 1.63, for each SD increase of the P-selectin value (233). In a study in Japan of 517 individuals, with a mean age of 57 years and with around 36% of participants having diabetes and 36% having hypertension, P-selectin was independently associated with both the presence of carotid artery plaques and with intima-media thickness (303). Thus, the associations between sP-selectin and both CACS and carotid artery plaques were known but strengthened by our study. The association with CACS may not have been significant after adjustments in our study because of a slightly lower power than the first study referenced above, or because of the dichotomization of CACS. Also, the mean CACS in the study by Bielinski et al was between 139 and 245 Agatston units, which is considerably higher than the mean CACS of 59 (not presented in the results) seen in our study and may have also influenced the results.

As described in the discussion of Study I, MH is also linked to both obesity and obstructive sleep apnea. Both of these conditions have also been linked to increased sP-selectin, and untreated
obstructive sleep apnea increases the risk of CVD (304). In a meta-analysis of 9 studies including 940 individuals of which approximately half had obstructive sleep apnea and half were controls, sP-selectin was higher in those with obstructive sleep apnea vs controls, even after matching for BMI (304). Obstructive sleep apnea involves intermittent hypoxia and sleep fragmentation, and this could increase sympathetic activity, low-grade inflammation, oxidative stress, platelet activity and endothelial dysfunction, thereby increasing sP-selectin levels and a prothrombotic state (304). Thus, the crude association between sP-selectin as a continuous value, and MH, could in part relate to these factors, as could the increased home BP in sustained hypertension.

In summary, the association between sP-selectin and sustained hypertension was novel, and was not substantially affected by adjustments for hsCRP, indicating that this association may not only be explained by inflammation. The association between hsCRP and WCH may indicate that the latter is not harmless. Our results also strengthen the already known association between both sP-selectin and hsCRP and atherosclerosis. To know whether these markers may be used in cardiovascular risk assessments, further longitudinal studies are necessary.

Study IV

We found that 100 mg of GA daily for 2 weeks suppressed both renin and aldosterone, and increased home BP. The results are consistent with pseudohyperaldosteronism, in which the glycyrrhetic acid in licorice inhibits activity in 11-ß-hydroxysteroid dehydrogenase type 2 in the kidneys (235), leading to increased cortisol concentrations and stimulation of the mineralocorticoid receptor (235, 305), increased sodium reabsorption and fluid retention, increased blood volume, and thus both increased BP and inhibition of renin secretion and therefore reduced renin and aldosterone concentrations, Figure 17. The increases in body weight and
NT-proBNP for a quarter of the participants are also consistent with increased blood volume. A previous study, albeit including only 2 individuals, has shown that the maximum plasma concentration of GA after intake of licorice is reached within 10 hours in humans (306). The timing of increased BP, with the first significant difference at day 3, is thus also compatible with an effect caused by licorice intake.

The mean 3.1 mmHg increase in systolic home BP during the licorice period is similar to the change in systolic home BP that has been seen with BP-lowering medications in individuals with slightly elevated baseline BP (96). Also, according to the Law of initial value (also Wilder’s principle), baseline values determine the degree of change (307), and it is therefore likely that the BP increase would be even higher if baseline values were higher, as in individuals with hypertension. Furthermore, even in individuals with baseline systolic BP as low as 115 mmHg, increased systolic BP has been linked to increased risk of stroke and all-cause mortality (308, 309). In fact, although evidence is lacking for initiating treatment below a systolic home BP of 135 mmHg (2), treatment titration down to a systolic BP of 120 mmHg is associated with reduced all-cause mortality (309). Thus, this 3.1 mmHg increase in systolic BP could be clinically relevant long-term. The increase in NT-proBNP for the quartile of participants with the most pronounced suppression of RAAS strengthens this assessment.

Although only numerically lower potassium levels were seen during licorice intake in our study, the effect was larger in the quartile of participants with the most pronounced suppression of RAAS, and licorice is known to cause potentially harmful hypokalemia, with increased risk of arrythmias, rhabdomyolysis and myopathy (235, 239). This may be an additional cause for concern, even though the effect on potassium levels would need to be verified in a larger sample.
Discussion

In summary, the results indicate that 100 mg of GA daily for 2 weeks stimulates fluid retention and thus increases body weight and BP. The change in BP could be considered clinically significant and harmful if it is sustained over longer time periods.

**Figure 17** (next page). Pathways of hormones in black and effects of glycyrrhizic acid in red, with numbers derived from the current study. Angiotensinogen is secreted from the liver, and it is cleaved by renin, which is secreted from the kidney, into angiotensin I (39). Angiotensin I is cleaved by angiotensin-converting enzyme (ACE) to angiotensin II (39). Angiotensin II acts as a vasoconstrictor and stimulates adrenal glands to secrete aldosterone (39). Aldosterone stimulates the kidneys to increase sodium reabsorption and thus fluid retention (39). Angiotensin II-receptor may also directly increase sodium reabsorption in the kidneys (310). Cortisol has the same affinity for the mineralocorticoid receptor as do aldosterone, but its concentration is 1000 times higher (235, 305). In the bowel, glycyrrhizic acid is metabolized into glycyrrhetinic acid (252), which inhibits the 11ß-hydroxysteroid dehydrogenase type II, thus increasing the cortisol concentrations in the kidneys (235) and the activation of the mineralocorticoid receptor. Illustration by Peder af Geijerstam. Abbreviations: NT-proBNP, N-terminal prohormone of brain natriuretic peptide.
Daily intake of 100 mg for 2 weeks

Glycyrrhizinic acid in licorice
- Metabolized in the bowel
  
Glycyrrhetic acid
- Inhibits activity
  
11-β-hydroxysteroid dehydrogenase type 2

Aldosterone from the adrenal glands

Cortisol
- Inactivates
  
Cortisone

Increased 3.1 mmHg

Renin from the kidneys

Angiotensin-converting enzyme (ACE) from the blood vessels

Angiotensinogen from the liver

Increased 204.1%

25% of participants

 Decreased 45.1%

Decreased 30.0%

Angiotensin I
- Cleaves
  
Angiotensin II
- Cleaves

Increased sodium reabsorption and fluid retention

Mineralocorticoid receptor

Stimulates activity

Increased blood volume

Blood pressure

Body weight

Increased 1.3%
Study IV Follow-Up (Ad-Hoc)

Due to a low participation rate, the results from the follow-up study on the participants with the most pronounced suppression of the plasma renin and serum aldosterone values were inconclusive. However, the numeric suppression of both plasma renin and serum aldosterone levels during the second period in all participants indicates that even daily intakes of licorice containing 50 mg of GA could suppress the RAAS activity. However, results of systolic and diastolic BP were not coherent, perhaps because of the high variability of BP measurements, and studies with sufficient power are needed to better answer the research question.
Ethical Considerations

All studies involved individuals who volunteered to participate. All studies also engaged participants by asking them to invest time and travel to complete the studies, and to participate in extensive testing. This testing entailed a risk of unexpected findings, which could contribute to improved care and health, but could also potentially produce findings which after further evaluation could turn out to be either non-treatable, or non-significant. Such findings may trigger unwanted concern or worry for the participant. Unfortunately, this is an unavoidable part of clinical research. However, participants were informed of these risks, and the risk of harm was minimized by only including testing which had the potential to answer the research questions and benefit the study aims. Additionally, for Study IV, we only include the number of participants needed to answer the research question based on a power calculation.

In Study IV, participants were also asked to ingest 2 different types of licorice. This entailed a theoretical risk of harm including allergic reactions as well as what has previously been reported in case reports: hypertension, hypertensive encephalopathy, hypokalemic myopathy, and cerebrovascular events (235, 239, 311). However, participants were young and healthy, evaluated for changes in BP and potassium levels, and asked to stop consumption and inform the investigators if they experienced any headache. Also, only individuals with normotensive BP at baseline were included in the study. Finally, the dose of licorice was low. Thus, much was done to minimize risk, and we assessed that the benefits of answering the research question outweighed the risks involved.
Methodological Considerations

Several reflections can be made regarding the methodologies used in the papers of this thesis, which may add nuance to the interpretation, strengths, and limitations of the results. First, general methodological topics concerning all studies will be discussed. Then, specific questions with regards to Study IV and Study IV Follow-Up will be addressed.

Measurements and Questionnaires

As exemplified throughout this thesis, BP is a highly complex measurement, and multiple challenges are present in all studies of BP. Determinants and factors to consider in the planning and interpretation of these studies include the choice of parameter (e.g., diastolic, systolic, pulse pressure, mean arterial pressure), the setting (at the office vs out-of-office, and attended vs unattended), the position (seated vs supine), the timing (morning vs evening, day vs night), the level of physical activity (at rest, moderate activity, peak exercise), the number of measurements (single, multiple, continuous), methodology and technique, and as with any measurement the population (e.g., sex, age, comorbidities, lifestyle choices, and medications). This multitude of factors are also challenging when comparing results to previous studies, as all factors are rarely the same.

In the studies presented in this thesis, the same validated BP device was used, and we used the same device to measure BP at the office and at home, since the use of different devices has been recognized as a limitation in previous studies (199). In studies I-III, the office BP was measured in the supine position whilst the home BP was measured in the seated position. In Sweden, it is not uncommon to measure office BP in the supine position (312), however, and the validity to the Swedish setting may therefore not have
been compromised. However, misclassification of BP status may have been introduced when compared to other studies. Studies on whether supine and seated BP differ have shown varying results (313–315). Finally, we did not use ambulatory BP measurements, which is again relevant to consider when comparing our results to other studies.

In all studies, home BP measurements were self-reported, thus giving room for reporting bias e.g., social desirability bias. In studies I–III, smoking status, as well as use of BP-lowering, lipid-lowering, and diabetes medications were also reported by the participants themselves through questionnaires, and we did not have access to register data to confirm prescriptions. Such questionnaire data could be influenced by recall, social desirability, and nonresponse bias (316), which may cause over- and underestimation. However, in a 2018 study of 10 244 individuals comparing self-reported medication use and registry prescription data, the reported use of BP-lowering medications, lipid-lowering medications, and insulin, had very good agreement with a Cohen’s kappa of between 0.87 and 0.92 (317). The study however could only measure dispensing rather than use of medications, which may differ significantly (317). Other studies on the same medication classes have showed a kappa between 0.55 and 0.96, thus varying greatly (317). For example, in a study of 7568 individuals, the agreement between self-reported medication and pharmacy data was between 0.69 and 0.81 for BP-lowering, lipid-lowering and oral diabetes medications (318). In conclusion, self-reported data may have introduced bias in our studies, and in particular with regards to the use of BP-lowering medication.

**Missing Data**

In studies I-III, missing data was less than 3% with one exception which is discussed later. In Study IV, all participants completed the study, and missing data was less than 3% for home BP
measurements, which may be considered low with regards to the more than 8 weeks of daily morning and evening measurements required by the participants, totaling 354 measurements and 1062 value registrations (systolic, diastolic, and heart rate) per participant (of which an average of around 10 measurements were thus missing). Of laboratory measurements in Study IV, 1 of 28 of plasma renin and serum aldosterone measurements, respectively, were missing during the second wash-out period.

Thus, missing data was overall low in all studies, and handled by listwise deletion i.e., omitting cases with missing data from analyses. Such handling assumes that values are missing completely at random, which is rarely if ever completely true (319). Although complete case analysis may introduce unknown bias and reduce power, imputation methods may also introduce bias (319). Furthermore, even when not missing completely at random, using more advanced methods to handle missing data rates below 5-10% may not improve results (280, 320).

The exception to the above was PWV in studies I-III, for which the missing rate was 30%. Pulse wave velocity was only evaluated if there was enough time to perform the analysis after other measurements had been performed. Thus, the missing could be non-random, influenced by factors that may delay examinations performed before PWV, such as echocardiography, which is more challenging to perform depending on body composition (321). Missing rates exceeding 40-60% may be difficult to impute and better analyzed as complete cases, as long as the limitation is discussed (280, 320). But high non-random missing rates below these values may preferably be handled using imputation of data (280), which could have improved our analyzes. In conclusion, missing values were low in general, but for PWV, imputation could have improved results.

Importantly, no statistical handling of missing data is perfect, and designing studies to avoid high rates of missing data is the
most important step to avoid such limitations (280). In the studies presented in this thesis, this could have been achieved by allowing more time for the collection of measurements (e.g., PWV), by automatic electronic transfer of measurement results (e.g., home BP measurements), by further facilitating the measurement process for the participants (e.g., offering evening or weekend laboratory measurements), and/or by issuing more frequent or timely reminders to participants when data was missing. Such changes would have reduced bias, but also in most cases increased the costs of the studies.

Statistical Tests

The $P$ value has been the topic of much debate, and the arbitrary and non-nuanced division into “significant” and “non-significance”, which lacks consideration of effect sizes and uncertainties, is discouraged by many (322). The use of the $P$ value may contribute to reporting bias, publication bias, “$P$-hacking”, “data dredging”, false positives and false negatives (323). Perhaps most importantly, the $P$ value is often misinterpreted, without consideration of its deductive aspect i.e., that it should be interpreted as the probability of observing the results if the null hypothesis was true. For example, if a study results in MH being more prevalent in men than women with $P = .03$, it does not mean that the probability of MH being more prevalent in men than women is 97%, but rather that there is a 3% probability of observing this result if in fact there is no difference between the sexes (323). Furthermore, the $P$ value expresses the agreement between the observed data and the predicted results provided that all assumptions of the statistical model are correct (322). Such assumptions e.g., random sampling, are practically challenging (322), and perhaps most often not fully met. A small $P$ value only conveys that the data is unusual if the hypothesis and all the other assumptions are true. Thus, it may be the result of a false hypothesis, but also
erroneous assumptions such as non-random sampling, loss to follow-up, or publication bias. Vice versa, a large $P$ value tells us that, provided that the hypothesis and all assumptions are true, the data is not unusual (322). In summary, interpreting the result requires weighting in the methodology leading up to the results, the sample size, the potential confounders, the results of previous studies, and potential mechanisms that could explain such results (or the opposite) (323). Such considerations are included in the discussion section of each paper.

Parametric analyses such as the $t$ test assume that the underlying data has a normal distribution, best characterized with the mean and standard deviation (324). With non-parametric analyses, such as the Wilcoxon rank sum test, distributions are compared without such an assumption of the distribution pattern (324). Although the sample size was large in the SCAPIS study, we made the distinction between using parametric and non-parametric tests depending on the sample distribution of each variable. This could be questioned based on the central limit theorem i.e., that if the sample size is large enough, the sampling distribution is approximately normal (325). Some statistical textbooks therefore advice that parametric tests may be used when the sample size is large enough e.g., at least 30 (326). Others argue that the benefit with parametric vs non-parametric tests on parametric data, compared with that of non-parametric vs parametric tests on non-parametric data, is less, and that non-parametric tests may be preferred (326). Furthermore, we used the non-parametric Wilcoxon signed-rank test in Study IV to evaluate the difference between the change in measurements of the 2 groups because the measurement values themselves were skewed. This choice may also be challenged with reference to the central limit theorem, by which change values will approximate to a normal distribution even though the distribution of the underlying absolute values is skewed.
Thus, an alternative approach would have been to use a paired samples $t$ test.

**Multiple Comparisons**

In all studies, tests were made to evaluate several different associations and outcomes. In each of studies I and II, 12 such tests were made, although only a few of them were intended to answer the primary research questions. We did not correct the results of these tests for multiple comparisons and thus, type I errors (false positives) may have been introduced. However, in Study III and IV, we used the Conover's many-to-one test with the Holm-Bonferroni method, and a Bonferroni corrected significance level, respectively, to adjust for the multiple comparisons.

The practice of adjusting for multiple comparisons has also been criticized. Epidemiologist Kenneth Rothman described it as an “insurance policy” against false positives, but at an unreasonably high cost of false negatives (327). He argued that such adjustments undermine the foundation of empirical research by which nature depends on laws, and that chance findings should indicate the need to learn more rather than to ignore (327), and implied that in fact, what will be explainable in the future may currently be considered random. Statistician Stian Lydersen critically questioned multiple comparison adjustments by asking whether the practice should then also apply to when the statistical tests are divided across multiple publications (328)?

**Categorization of Data and Logistic Regression**

Categorization of continuous variables results in loss of power and loss of heterogeneity within categories (329, 330), reduces the ability to discover non-linear relationships, and produces results which indicate relative and not absolute differences between groups. Dichotomization, too, may increase variability of the
Methodological Considerations

estimate and decrease power, but may be a deliberate choice because of a desire to align with previous research, to compensate for a skewed distribution, or to simplify the presentation of results (331). Dichotomization also removes the potential to discover dose-response relationships, which can otherwise support an association (332). Furthermore, odds ratios are scaled by a factor which depends on the variables used in the regression model, so that when models use different explanatory variables, their resulting ORs cannot be compared or used as is in a meta-analysis (333).

In Study III, we choose to categorize inflammatory markers as quartiles because of their skewed distribution. Clinical or otherwise established cut-offs would have been an alternative, but there were none that we knew of for sP-selectin and consistency in the categorization of the independent variables was prioritized. An alternative would have been to perform a linear regression after log transformation of the data, but such transformation obscures the absolute values of the variables which may complicate interpretation. To counter the disadvantages of dichotomization, we performed additional linear regressions of each inflammatory marker as a continuous variable vs the cumulative prevalence of each hypertension phenotype. However, this analysis did not compare each hypertension phenotype to sustained normotension, and thus was not directly comparable to the logistic regression results. However, the (lack of) overlap of the confidence intervals of each hypertension phenotype vs sustained normotension indicate that results were comparable to those of the logistic regressions.

Adjusting for Confounders

In studies I-III, we adjusted for several potential confounders, including age and sex. A confounder is a variable associated with both the independent and dependent variable, yet not as a mediator (316). If unadjusted for, results could show either a false relationship or mask a true relationship (280). By using multivariable
regression analysis, confounders are statistically held constant with the aim to isolate the effects of the independent variable (316). All variables which may constitute risk factors for the dependent variable are potential confounders, and sex and age are thus almost always considered (280). Although this approach is common and one of several methods to adjust for confounders (316), the method has also attained some criticism. For example, Shapiro et al argues that instead of controlling for sex, it should be treated as an important aspect to the research question (334). Subgroup analyses would thus be an alternative.

Controlling for confounders may also give the impression that the relationship hypothesized is causal, or that the adjustments have removed all confounding (measured and unmeasured), both of which are untrue. However, this was discussed in the limitations and could also be considered a basic understanding of statistics required by the reader. Finally, by also presenting the crude, unadjusted results, the reader may at their own discretion assess both results.

Association and Causation

Studies I-III were cross-sectional, and we can therefore not draw any conclusions with regards to neither longitudinal associations nor causality.

However, Study IV was a randomized controlled trial, in which participants were randomly assigned to the exposure, and the effects on the outcome can therefore be considered causal. This however assumes a flawless study design. A crossover design only controls for constant confounders (such as sex), but not those which may change over time (such as diet) (332). Also, the study was not blinded, and thus bias could be introduced and challenge causality if for example participants knew when they were given the intervention vs control substance and changed behavior or reporting of
results accordingly. When studying whole licorice, blinding is practically not possible as the taste difference between licorice and salty licorice, despite their similarities, is noticeable. However, we consider this risk as limited, and that the results on BP measurements are corroborated by the blood samples which we also included. Finally, using isolated GA (e.g., in capsules, which would enable the use of placebo capsules as control) would not be comparable to using whole licorice, as isolated GA has higher bioavailability (306, 335). However, studies of the bioavailability of licorice have been small and it is not clear if licorice GA concentrations were measured (306).

External Validity

All study participants were from the county Region Östergötland in Sweden. This region may differ or be an outlier in some demographic or other health related factors, which could affect results. For example, Region Östergötland had the highest relative unemployment rate in Sweden in 2023 (336). External validity in its strictest sense may thus be limited to this population. However, it is plausible that most results and conclusions could also apply to populations with similar demographics. For results which have already been seen in studies of different populations, our results broaden the external validity.

The first 3 studies used data from the SCAPIS cohort, which is one of the largest cohort studies of its kind, including advanced examinations of cardiovascular markers, including imaging. The size of the cohort, and the general data quality with low missing rates and thorough documentation and standards of examinations, is a major strength of the studies. However, participants were aged 50-64 years, and thus external validity is limited to similar age groups. Also, despite a large study sample, the participation rate was 58% which could be the result of self-selection and challenge the samples representativeness. In the SCAPIS pilot study, participation
was associated with socioeconomic status, but there was no difference between those that choose to participate or not in terms of known diabetes or CVD (337).

In Study II, only participants that did not use BP-lowering medications were included. The reasoning behind this inclusion criteria was that MH in individuals with a known diagnosis of hypertension is regarded as a separate entity (often referred to as uncontrolled masked hypertension).

In Study III, only participants with inflammatory markers below certain levels were included. The reasoning behind this inclusion criteria was to exclude those with inflammation due to other processes, such as infectious disease, which could distort the potential associations between inflammation and CVD which we aimed to investigate. Unintentionally, this may have also affected the findings with regards to other aspects related to inflammation, such as obesity which is known to be associated with higher CRP levels (338).

In Study IV, the participants were young adults and healthy, which limits the generalizability of the results. However, there is no plausible reason to believe that the effect of licorice would be less harmful for a population of older age, or with comorbidities. Rather, the opposite could be true because of decreased cardiovascular reserves to manage the excess fluid, and this was in fact a major reason for our choice of inclusion criteria. Thus it would be reasonable to exert even greater caution with licorice consumption in older or comorbid populations until more research is available.

Study IV and Study IV Follow-Up (Ad-Hoc)

In Study IV, we performed a power calculation to reach an appropriate sample size before commencing the study, which is important to avoid too small cohorts with the risk of type II errors (false negatives) or too large cohorts with unnecessary costs and
risk exposures (339). The power is the probability before a test is performed that it will reject the null hypothesis when the alternative hypothesis is correct (280), and we choose the most commonly used level of 80%. We then used an assumed mean systolic BP of 120 mmHg, a standard deviation of 5 mmHg, and a clinically meaningful difference of 4 mmHg.

Of the 28 participants, the mean systolic home BP was 106.5 mmHg, the standard deviation was 9.5 mmHg, and the detected difference was 3.1 mmHg. The lower difference detected compared to the one used in the power calculation is not unreasonable given that an 80% power to detect a 4 mmHg difference would also entail the possibility to detect a lower difference, albeit with less probability of doing so. We did not include the secondary or exploratory aims in the power calculation, which could have ensured a certain probability to detect a meaningful change in e.g. body weight.

The duration of each of the intervention, control, and washout periods was 2 weeks. This was chosen to cover the time until a BP response would likely occur and have normalized, respectively, based on previous studies, while considering practical limitations for participants. The design entailed a total of 354 BP measurements for each participant, and a longer duration would likely have increased both missing and drop-out rates. Effects beyond the 2 weeks, whether sustained, increased or decreased, could not be determined. Also, the diastolic BP did not return to baseline during the 14 day post-intervention washout period, which could indicate a carry-over effect (i.e., that the washout period was too short).

Results were at first presented as the changes in absolute values e.g., for weight the change in kg, but was changed to percentages by request of the reviewers. Absolute values are easier to interpret relative to clinical significance such as threshold levels, but percentages are easier to interpret relative to baseline.

We used a paired Wilcoxon signed-rank test instead of a more advanced linear mixed-effects model. The latter can account for
both fixed (such as intervention vs control) and random effects (such as sampling block), and thus be used to analyze e.g. carry-over effects, but the paired Wilcoxon signed-rank test is more common and we were more confident in the performance of that analysis, which we therefore prioritized (340). Finally one-sided tests are recommended if the direction of change of the hypothesis is clear before the analysis is performed (322). Although this was true for several of the analyzes in Study IV based on previous studies, we choose to use 2-sided tests because it is more common, which increases credibility and thus outweighs the potential benefits of reducing the type II error rate.

The ad-hoc Study IV Follow-Up had only 3 participants, and thus insufficient power, to determine any associations. The results may at most be regarded as hypothesis generating, and larger studies are necessary to draw any conclusions. According to the regression to the mean phenomenon, subsequent measurements of individuals or groups with extreme measurements (in relation to the mean) will likely be less extreme (97). In line with this, it is likely that the extreme response to licorice intake in these individuals in Study IV was the result of both an increased sensitivity to the substance, and a chance factor. The latter would likely be attenuated in subsequent measurements. It could therefore be argued that the true most sensitive quartile of participants would be a slightly different one than the one we selected, and that the true most sensitive quartile would also display an even more pronounced response to a daily GA intake of 20 or 50 mg than what we observed in the ad-hoc study.
Conclusions

Hypertension is the most common preventable cause of premature all-cause mortality, and the value of improved diagnostics and treatment is therefore considerable. Out-of-office compared with office BP measurements are known to better predict cardiovascular mortality.

In Study I, we confirmed the known association between MH and diabetes and strengthened the association with dysglycemia by also evaluating the continuous relationship between HbA1c and the systolic office-home BP difference. In Study II, we confirmed the association between MH and higher BMI, higher hsCRP as a marker of inflammation, and higher PWV as a marker of arterial stiffness. Furthermore, we could also show for the first time that MH was associated with an increased risk of CACS. In Study III, we showed that both sP-selectin and hsCRP were increased in all hypertension phenotypes. The association between sP-selectin and sustained hypertension was novel, and was not substantially affected by adjustments for hsCRP, indicating that this association may not only be explained by inflammation.

The results imply that home BP has a greater relative value for individuals with dysglycemia, starting already at prediabetes levels, as well as for men and individuals with obesity. Furthermore, the results strengthened the already recognized link between MH and increased cardiovascular risk. Our results also confirm previously known associations between inflammation, hypertension, and CVD. We also found that the association of sP-selectin and hypertension may not be only inflammatory, and further research, including other cohorts and inflammatory markers, could advance this understanding. The results also suggest that, although home vs office BP may be more strongly associated with increased cardiovascular risk when they are compared singularly, office BP may
have an additive value to that of home BP so that the combination of office and home BP is superior to any one of them. However, the cross-sectional nature of these studies is an important limitation, and neither longitudinal associations nor causations can be determined. Longitudinal studies and randomized controlled trials are necessary to better understand how these factors and home BP relate over time.

The results of Study IV indicate that even in young and healthy individuals, consuming a low dose of licorice containing 100 mg of GA results in pseudohyperaldosteronism with increased BP, and that for the individuals with the most pronounced reaction, the resulting excess blood volume may also increase body weight and strain the heart.
Clinical Implications

Clinically, in conjunction with previous findings, the current studies indicate that using home BP in addition to office BP may be more relevant in individuals with known CVD risk factors, such as male sex, diabetes, and obesity, as well as in individuals with end-organ damage such as arterial stiffness and incident cardiovascular events in spite of normal and supposedly well controlled office BP. The utilization of home BP measurements in Swedish primary care is low and may need to be promoted (312). However, whether the increased prognostic information of home BP also translates into benefits from treating towards a normalization of it is not known. There is a need for longitudinal and eventually randomized controlled trials on the value of using home BP in the diagnosis and treatment of hypertension.

The results of the study on licorice brings into question the current recommendations of both the WHO and the European Union suggesting that 100 mg of GA is probably safe, and the recommendations of the Swedish Food Agency suggesting a safe limit of 50 grams of licorice (239-241). However, it may be challenging for consumers to understand the GA-content of licorice products currently on the market. For example, in a Danish study from 2023 analyzing 219 licorice product samples, as little as 83 mL of tea, 59 grams of ice cream, or 4.3 grams of confectionary contained 100 mg of GA, yet 10% of the licorice samples analyzed were not correctly labeled (242). The concentration of GA also varies with season and plant characteristics, and in a 2022 US study, 38% of licorice products were mislabeled in terms of species (236, 243). In our study, the product we used had a 50% higher GA content than specified on the label, and only 3.3 grams was sufficient to contain 100 mg of GA (31).
The warning label in the European Union, mandated only for products with a GA concentration above 4 grams per kilo (242), means that products containing 0.4% GA are not labelled, despite only 25 grams of such products being sufficient to reach the 100 mg GA threshold. Thus, even the tea and ice cream samples with the highest GA concentrations analyzed in the Danish study would not have to carry the warning label mandated by the European Union, and consumers would themselves have to be aware that such small amounts could be harmful.

In summary, the results suggest a need to lower the recommended daily amount of GA, to improve labelling of licorice products, and to nuance the recommendations so that they also convey the relative risk with even small amounts of licorice for long periods of time. To mandate producers of these products to regularly measure the GA content, and to include it in the labelling of the product in a way that is easy for the consumer to comprehend (for example, the number of grams that equals 100 mg of GA), could also help consumers to make healthier choices.
Future Research

This thesis confirms several associations between home BP and markers of cardiovascular risk. As discussed in this thesis, out-of-office BP also better predicts cardiovascular mortality (123). Furthermore, MH is associated with an increased risk of cardiovascular events (103, 170, 171, 341). Despite this, office BP is the most studied and used methodology for the purpose of diagnosing and treating hypertension (2). And although the stronger association of home vs office BP and CVD is established, no study to date has evaluated whether treating hypertension to target a lower home rather than office BP would further reduce incident major adverse cardiovascular events or all-cause mortality in a population (2). In the 2023 ESH Guidelines for the management of arterial hypertension, this lack of randomized controlled trials on treatment of home BP is mentioned as a “crucial gap” (2). Thus, this is a key research question for the scientific community to answer in the years to come.

Regarding home BP in general, physicians report difficulties regarding inconsistent methodology amongst patients, transfer of results from patients to physicians, and interpretation of results, and that online education, and digital systems to integrate home BP measurements with the medical records used by physicians, could mitigate some of these issues (342). Whilst not a focus of the current thesis, to increase the value of home BP measurements, it would also be of value to study strategies to mitigate these questions.

Similar to the progression from office to home BP measurements, wearables such as watches and wristbands that are even more portable and could enable near continuous BP measurements, are under development (343). It is possible that such new devices could also further advance the portability of BP
measurements. However, these devices have yet to show sufficient reliability (344), and further research is needed.

On licorice, it is necessary that at least one independent research group confirms our results in order for conclusions to be made. Further research is also needed to understand whether even smaller amounts of GA, and/or for longer time periods, affect BP. To inform authorities, studies on licorice consumption in various communities to determine the cost-effectiveness of improved labelling or work to increase awareness could also be of value.

Finally, the use of home BP in these studies was cost-efficient, used little valuable clinician time and yielded crucial measurements. This indicates that using home BP in research can be efficient both in terms of cost, power, and precision.
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Papers

The papers associated with this thesis have been removed for copyright reasons. For more details about these see:

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