UNIVERSITY OF OSLO
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THE RISK OF DEVELOPING DIABETES IN HYPERTENSION

-Insulin sensitivity, AT-1 receptor blockade and sympathetic activity

Tonje Amb Aksnes
Ullevål University Hospital

ULLEVÅL University Hospital

HELSE ØST

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2. Abbreviations

ACEI	angiotensin-converting enzyme inhibitor
ARB	angiotensin II-receptor blocker
BMI	body mass index
BRS	baroreflex sensitivity
BP	blood pressure
CCB	calcium channel blocker
CHD	coronary heart disease
CV	coefficient of variation
CVD	cardiovascular disease
DM	diabetes mellitus
ECG	electrocardiogram
GDR	glucose disposal rate
HDL	high-density lipoprotein
HF	high frequency
HOMA-IR	homeostasis model assessment for insulin resistance
HR	hazard ratio
HRV	heart rate variability
hs-CRP	high sensitivity C-reactive protein
IL-6	interleukin 6
LF	low frequency
LVH	left ventricular hypertrophy
NN	normal-to-normal
NS	non-significant
PAI-1	plasminogen activator inhibitor type 1
PPAR-γ	peroxisome proliferator-activated receptor-γ
RAS	renin-angiotensin system
RAAS	renin-angiotensin-aldosterone system
r_s	Spearman's correlation coefficient
SDNN	standard deviation of all NN intervals
SEM	standard error of the mean
SNS	sympathetic nervous system

TNF-α	tumor necrosis factor-alpha
VLF	very low frequency
WBV	whole blood viscosity
WHO	World Health Organization

3. List of papers

Paper I: Aksnes TA, Kjeldsen SE, Rostrup M, Størset Ø, Hua TA, Julius S. Predictors of new-onset diabetes mellitus in hypertensive patients: the VALUE trial. J Hum Hypertens 2008; 22(8):520-7.

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Paper IV: Aksnes TA, Flaa A, Sevre K, Mundal HH, Rostrup M, Kjeldsen SE. Effect on plasma noradrenaline may explain some of the improved insulin sensitivity seen by AT-1 receptor blockade. Blood Pressure 2008; 17(3):156-63.

Paper V: Aksnes TA, Kjeldsen SE, Rostrup M, Omvik P, Hua TA, Julius S. The impact of new-onset diabetes mellitus on cardiac outcomes in the Valsartan Antihypertensive Long-Term Use Evaluation (VALUE) trial population. Hypertension 2007; 50(3): 467-73.

4. Introduction

4.1 Hypertension

4.1.1 Epidemiology and classification

Hypertension as in "high tension" and "high blood pressure" is a disease affecting billions of people worldwide. Essential hypertension can be defined as a rise in blood pressure of unknown cause that increases the risk for cerebral, cardiac, and renal events[1]. In a recent review the lifetime risk of becoming hypertensive in industrialised countries was estimated to exceed 90%[1]. Historically more emphasis was placed on diastolic than on systolic blood pressure as a predictor of cardiovascular morbidity and mortality[2], and systolic blood pressure limits was not included in early guidelines and hypertension trials. In recent years more emphasis has been on the observation that in especially elderly people the risk of morbidity and mortality is directly proportional to the systolic blood pressure and the pulse pressure (systolic blood pressure minus diastolic blood pressure)[3]. In a large meta-analysis of observational data from 61 studies in almost 1 million subjects without cardiovascular disease, both systolic and diastolic blood pressures were independently and similar predictive of vascular (and overall) mortality, without any evidence of a threshold down to at least 115/75 mmHg[4], and therefore risk assessment in current guidelines are based on both systolic and diastolic measurements[3, 5-7].

According to the new European Guidelines from the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC), hypertension is graded from 1-3 of values above 140/90 mmHg (Table 1)[3]. However, the real threshold for hypertension and treatment must be considered flexible, being higher or lower based on the total cardiovascular risk of each individual patient[3].

Table 1. Definitions and classification of blood pressure levels (mmHg) according to ESH/ESC 2007 guidelines[3].

Category	Systolic Blood Pressure		Diastolic Blood Pressure
Optimal	<120	and	<80
Normal	120-129	and/or	80-84
High normal	130-139	and/or	85-89
Grade 1 hypertension	140-159	and/or	90-99
Grade 2 hypertension	160-179	and/or	100-109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension	≥140	and	<90

**** When a patient's systolic and diastolic blood pressures fall into different categories the higher category should be applied***

4.1.2 Pathophysiology

Hypertension is a disorder of mismatch between intravascular volume and vasoconstriction resulting in excessive wall stress that damages the blood vessels and organs. The pathogenesis of essential hypertension is incompletely understood, and involves complex interactions between genetic, environmental and demographic factors[8]. Major pathophysiological mechanisms include increased sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) activity[9], but a variety of other neural, hormonal, vascular and metabolic factors are also involved.

The SNS and renin-angiotensin system (RAS) have both mutually reinforcing actions that combine to regulate blood pressure. Sympathetic drive increases renin secretion from the juxtaglomerular cells of the kidney, thereby exerting amplified effects on the RAS, and angiotensin II increases noradrenaline release from sympathetic nerve terminals and potentates the vasoconstrictor responses to noradrenaline[9]. Blood pressure is regulated in the long-term by adjusting blood volume through urinary sodium and water excretion by the kidneys[10] and short-term regulation is exerted through hormones, mechanical factors and neural reflexes[11]. The arterial baroreceptors are mechanosensitive nerve endings sited in the carotid sinuses and the aortic arch and provide an important and powerful feedback mechanism of blood pressure regulation[12]. Although the pathophysiology may be complex, the result is an increase in blood pressure known to make organ damage and increase morbidity and mortality.

4.1.3 Complications

Hypertension has been called the "silent killer"[13] as many do not experience any symptoms of the high blood pressure itself. However, the World Health Organization (WHO) has reported

high blood pressure as a leading cause of death worldwide[14]. Stroke has been labelled as the most important "hypertension related complication"[2, 3] , but also coronary events, heart failure, peripheral artery disease and end stage renal disease are known as important hypertension related diseases. As patients with hypertension often exhibit additional cardiovascular risk factors like dyslipidaemia, glucose metabolism abnormalities, obesity or left ventricular hypertrophy[15], the assessment of total cardiovascular risk is important when evaluating and treating these patients[3].

4.1.4 Treatment

Non-pharmacological treatment regimens like low salt diet, weight loss, exercise, and alcohol restriction have been shown in meta-analyses to lower blood pressure[16-19]. However, antihypertensive drugs often have to be used to reach the blood pressure target of below 140/90 mmHg (or lower if high-risk patients). Five major classes of antihypertensive agents are recommended according to recent guidelines, either in monotherapy or in suitable combinations; thiazide diuretics, calcium channel blockers (CCBs), angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II-receptor blockers (ARBs) and beta-blockers[3]. Most patients require multiple drugs to achieve blood pressure targets, but unfortunately many patients still remain untreated or under-treated.

4.2 Diabetes mellitus

4.2.1 Epidemiology and classification

The global number of individuals with diabetes mellitus in 2000 was estimated to be 171 million (2.8% of the world's population), but many patients are undiagnosed and the prevalence is increasing exponentially primarily because of increase in sedentary lifestyle and obesity[20]. The estimated lifetime risk of diabetes mellitus is 30-40%[21], and as in most other countries in the world the prevalence of diabetes mellitus in Norway is also increasing[22].

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects of insulin secretion, insulin action, or a combination of both[23]. It consists in two distinct forms; Type 1 which usually occurs in younger subjects and type 2 which is far more common and comprising over 90% of adults with diabetes mellitus[23].

There are different diagnostic criteria and different ways to classify dysglycaemia and diabetes mellitus, but the WHO 1999 criteria, as shown in Table 2, is often used[24]. Progression from normal glucose tolerance to diabetes mellitus involves intermediate stages.

Impaired fasting glucose (IGF) and impaired glucose tolerance (IGT) are both known risk factors for later diabetes mellitus development[25], but IGT is probably a stronger marker of future cardiovascular disease[25-27].

Table 2. Criteria used for glucometabolic classification according to WHO 1999[23, 24]

Glucometabolic category	Classification criteria*
Normal glucose regulation	Fasting plasma glucose <6.1 mmol/L + 2-hour post-load plasma glucose < 7.8 mmol/L†
Impaired fasting glucose (IFG)	Fasting plasma glucose ≥6.1 mmol/L and <7.0 mmol/L + 2-hour post-load plasma glucose <7.8 mmol/L†
Impaired glucose tolerance (IGT)	Fasting plasma glucose < 7.0 mmol/L + 2-hour post-load plasma glucose ≥7.8 mmol/L and <11.1 mmol/L†
Impaired glucose homeostasis	Impaired fasting glucose or impaired glucose tolerance
Diabetes mellitus	Fasting plasma glucose ≥7.0 mmol/L or 2-hour post-load plasma glucose ≥11.1 mmol/L†

**** 1 mmol/L glucose= 18 mg/dL glucose***
† Standardised oral glucose tolerance test (OGTT); Performed in the morning, after an overnight fast (8-14 h); one blood sample should be taken before and one 120 min after intake of 75 g glucose dissolved in 250-300 mL water in the course of 5 minutes

4.2.2 Pathophysiology

Hyperglycaemia results from insulin supply insufficient to meet the body's needs, and in diabetes mellitus the hyperglycaemia exceeds the threshold where the risk of diabetic retinopathy is currently thought to begin (Table 2)[24]. In type 1 diabetes mellitus there is a lack of pancreatic insulin production due to β-cell destruction and an absolute insulin deficiency[23]. While in type 2 diabetes mellitus there is a chronic and often progressive peripheral insulin resistance and an insufficient insulin supply due to increased demands and a relative insulin deficiency. These processes antedate the clinical diagnosis of diabetes mellitus, they cause the disease, and they continue to worsen after the diagnosis is made. The rising blood glucose in type 2 diabetes mellitus results from a combination of genetic predisposition, unhealthy diet of energy dense food, physical inactivity, and increasing weight with a central distribution resulting in complex pathophysiological processes[23]. In the following I will focus on type 2 diabetes mellitus.

4.2.3 Risk factors

To qualify as a risk factor for diabetes mellitus, the association between the risk factor and the disease must be independent of known confounders and the evidence must suggest that interventions to reduce the risk factor (not always possible) will lead to a reduction in risk of diabetes mellitus[25]. Early detection and treatment of risk factors may decrease the chance of

developing diabetes mellitus and diabetes complications. The entity of risk factors named the metabolic syndrome, as discussed below, are known to increase the risk of developing type 2 diabetes mellitus[28].

Possibly, the most important risk factor for diabetes mellitus development may be genetics. A first-degree relative with diabetes mellitus is important when assessing a patient's risk of developing diabetes mellitus. There are also high-risk populations and known differences in diabetic risk between races e.g. African Americans are known to have an elevated risk of diabetes mellitus compared with Caucasians[29, 30]. The risk for diabetes mellitus also increases with age[31].

When looking at more modifiable risk factors, dysglycaemia and increased blood glucose are as expected important risk factors for diabetes development[32]. IFG as defined above is a known risk factor for diabetes mellitus[33], but there is probably a continuum of increased risk from even lower levels of "normal" fasting glucose. The results from the MELANY study showed that increased fasting glucose level from even within the normal range (<5.55 mmol/L) constitutes an independent risk factor for later type 2 diabetes mellitus development[34]. These results may suggest that there is a relative overproduction of hepatic glucose already existent in patients at risk of diabetes and in a retrospective analysis there has been shown that there is an elevated risk of cardiovascular disease (myocardial infarction and stroke) more than 15 years before the clinical diagnosis of diabetes mellitus[35]. Unhealthy diet, smoking, physical inactivity and obesity are other important modifiable risk factors for diabetes[36]. Some data have shown that waist circumference, as a measurement of obesity, predicts diabetes mellitus marginally better than body mass index (BMI)[37-39], however other studies have shown equivalent predictive value[38]. Low socioeconomic status is also shown to be an important risk factor for diabetes mellitus[31].

Hypertension and diabetes mellitus often cluster together, and other cardiovascular diseases like peripheral vascular disease also increase the risk of diabetes development[31, 40]. Blood pressure and blood pressure progression were strong and independent predictors of type 2 diabetes mellitus in The Women's Health Study[41]. The multivariable adjusted hazard ratio (HR) was 2.03 (1.77-2.32) in patients with hypertension compared to patients with normal blood pressure (120-129/75-84 mmHg) after adjusting for BMI and other components of the metabolic syndrome[41]. This is in line with the results from the ARIC study showing a relative risk of developing diabetes mellitus of 2.34 (2.16-2.73) in hypertensives[42].

Different treatment regimens (e.g. diuretics, beta-blockers, and steroids) and other biochemical markers (e.g. dyslipidaemia, ALAT (alanine aminotransferase), and CRP (C

reactive protein)) have been linked with diabetes development, but the association may not be so general and independent.

4.2.4 Complications

Diabetes mellitus is associated with development of specific long-term organ damages including retinopathy, nephropathy and autonomic dysfunction[23]. These microvascular damages are related to the hyperglycaemia and the threshold for the diabetes diagnosis[24]. Patients with diabetes mellitus are also at a particularly high risk for cardiovascular, cerebrovascular, and peripheral artery disease[23], and these macrovascular diseases are with lesser degree associated with hyperglycaemia and the increased risk starts below the level of blood glucose used to define diabetes mellitus and before the actual diabetes mellitus diagnosis[25, 43]. More than 20% of patients admitted for suspected myocardial infarction have type 2 diabetes mellitus[44]. And a difference in morbidity and mortality between patients with and without diabetes mellitus has remained despite improved therapeutic modalities that have resulted in a decline in the overall morbidity and mortality following acute coronary artery disease[45]. Possible mechanisms may be diffuse coronary atherosclerosis, diabetic cardiomyopathy, autonomic neuropathy, increased heart rate, increased thrombus formation, or an impaired fibrinolytic function in diabetics[44]. In long-term follow-up studies it has been shown that patients with diabetes mellitus without any prior myocardial infarction have similar risk for fatal coronary heart disease as non-diabetic patients with prior myocardial infarction[46-48]. The majority of deaths in patients with diabetes mellitus result from accelerated cardiovascular and cerebrovascular atherosclerosis[25], and cardiovascular mortality is increased 1.5-4.5-fold, and all-cause mortality is increased 1.5-2.7-fold in diabetics[25]. The combination of diabetes mellitus and hypertension may have especially ominous consequences and increases the risk of coronary heart disease independently and dramatically[49, 50].

4.2.5 Treatment

Type 2 diabetes is a progressive disease and prevention or treatment requires modification of the underlying condition and reduction of the hyperglycaemia. Lifestyle intervention is important and has shown to reduce the risk of developing diabetes in patients with IGT with almost 60%[51, 52], and lifestyle intervention with diet and physical activity should be emphasised in all patients. Oral anti-diabetic drugs (e.g. acarbose, metformin, sulfonylurea, thiazolidinediones) may reduce hyperglycaemia due to reduced glucose absorption, increased

insulin sensitivity and increased insulin secretion. Many patients with type 2 diabetes mellitus may also need insulin treatment to get their diabetes under control. Aggressive management of other cardiovascular risk factors including hypertension and dyslipidaemia are also important and tight blood pressure control has shown to substantially decrease the risk of diabetes-related deaths and the progression of microvascular and macrovascular complications[53, 54]. Some antihypertensive treatment regimens has also shown potential to reduce or postpone diabetes development[55, 56].

4.3 Insulin resistance

In healthy subjects insulin action and secretion are coordinated to regulate the blood glucose level into normoglycaemia. The response to elevated plasma glucose is insulin secretion that stimulates glucose uptake and glycogen synthesis and inhibits glycogenolysis and gluconeogenesis in insulin responsive tissues (i.e. liver, fat and skeletal muscle). Skeletal muscle is the largest tissue by mass regulated by insulin and is responsible for more than 80% of insulin stimulated glucose disposal[57]. The glucose uptake in peripheral tissue is a complex mechanism. The activation of the glucose-transport system is highly regulated starting with insulin stimulation and via series of intracellular proteins resulting in translocation of the glucose transporter GLUT-4 to the sarcolemma membrane where glucose transport takes place via a facilitative diffusion process[58]. Glucose transport into muscle can also be stimulated by insulin-independent mechanisms activated by contraction and hypoxia, but less is known about these mechanisms[58].

The term insulin resistance refers to reduced capacity of insulin to stimulate glucose uptake and utilisation, and is a primary defect leading to the development of pre-diabetes and overt type 2 diabetes mellitus[58]. In insulin resistance, intracellular defects in the insulin signalling sequence result in reduced GLUT-4 translocation and glucose uptake. Skeletal muscle accounts for a large part of insulin-stimulated glucose disposal and is the major site of peripheral insulin resistance. The aetiology of skeletal muscle insulin resistance is multifactorial, but accumulating evidence shows that over-activity of RAS is one important contributor[58]. Other mechanism like oxidative stress, SNS activity, and excessive visceral adipose tissue lipolysis or reduced adiponectin levels may also contribute[59]. A compensatory hyperinsulinaemia is thought to permit normal glucose tolerance as long as pancreatic β-cell function is sufficient.

A number of methods have been developed for the quantitative measurement of insulin sensitivity. The hyperinsulinaemic glucose clamp technique is considered to be the

most accurate or the "gold standard"[60], but other methods like insulin infusion sensitivity test and different model assessments also exist[61, 62].

Insulin resistance is associated with dyslipidaemia, hypertension, hypercoagulability and atherosclerosis. The link between insulin resistance and hypertension is not known for sure[63, 64], as only about 50% of hypertensive subjects are insulin-resistant[64]. Post-insulin-receptor defects, altered skeletal muscle fibres and decreased skeletal muscle blood flow with reduced delivery of insulin and glucose may cause insulin resistance in hypertensives[63]. Insulin resistance in hypertension appears to be strongly dependent of abdominal and overall obesity[65], but not entirely[66]. According to the hypothesis of Landsberg[67], insulin resistance in the obese is a mechanism evolved to limit further weight gain, and the price to pay is the hyperinsulinaemia and sympathetic activation which increase blood pressure through vasoconstriction, kidney sodium reabsorption and increased cardiac output. According to the hypothesis of Julius[68], enhanced sympathetic activity is the primary factor to be associated with both hypertension, insulin resistance and possibly obesity. Sympathetic influences may reduce insulin sensitivity via haemodynamic effects due to vasoconstriction and increased diffusion target-distance or by direct cellular effects[59]. Insulin resistance can reciprocate sympathetic stimulation and sympathetic stimulation can cause insulin resistance, and a vicious cycle may evolve in which the components reinforce each other[68] and a classic "chicken and egg" question is raised[59].

4.4 The metabolic syndrome

In 1988 Reaven described a syndrome designed "syndrome X" based on the clustering of the following abnormalities: resistance to insulin-stimulated glucose uptake, hyperinsulinaemia, hyperglycaemia, increased triglycerides, decreased high-density lipoprotein (HDL) cholesterol and high blood pressure[69]. The clustering had been known for decades[70], but he proposed insulin resistance as the common feature and the aetiology of the syndrome. However, growing evidence suggests that several of the factors are primarily caused by obesity (especially intra-abdominal adiposity or visceral obesity) and the terms "metabolic syndrome" or "cardiometabolic syndrome" are now more commonly used[71]. Others again look at the SNS as the 'primum movens' of these cardiovascular and metabolic alterations[59]. A lot of other features and more "non-traditional" risk factors have also been discussed to be included in the syndrome like dysfunction of inflammation, coagulation, fibrinolysis, platelets, lipoproteins, endothelium, and miscellaneous biological processes. Currently, there are different definitions of the metabolic syndrome proposed by international and national

organisations or expert group as shown in Table 3. And there is an ongoing discussion about the syndrome's existence. The debate is (in part) related to lack of a universally accepted definition, but also due to the aetiology and doubts regarding the need for these disparate cardiovascular risk factors to be "lumped" together under one "artificial" diagnostic heading. All individual metabolic syndrome components have been shown to be independent risk factors for cardiovascular disease and death, so it's not surprising that the clustering of these abnormalities has been reported to be accompanied by a substantial increase in the incidence of coronary disease as well as overall cardiovascular morbidity, cardiovascular and all-cause mortality[59]. However, patients known to have this clustering of abnormalities have increased cardiovascular risk and an increased risk of developing diabetes mellitus[28, 72-75], whether or not this is due to the clustering or the individual components themselves.

Table 3. Different definitions for metabolic syndrome

	WHO (1999)[24]	**EGIR (1999)[76]**	**NCEP /ATPIII (2001[77] and *2005 update[78])**	**IDF (2005)[79]**	**ACE /AACE[80]**
Main criteria	Insulin resistance *or* DM/IGT/ IFG≥6.1mmol/L	Insulin resistance	**3 of the following:** • Abdominal obesity (>102(♂)/ 88(♀) cm)* • TG≥1.7 mmol/L • HDL<1.0(♂)/ 1.3(♀) mmol/L • BP≥130/85 mmHg *or treatment* • IFG≥6.1(*5.6)-6.9 mmol/L *or treatment*	Central obesity (waist ≥94(♂)/ 80(♀) cm)	• TG≥1.7 mmol/L • HDL<1.0(♂)/ 1.3(♀) mmol/L • BP≥ 130/85 mmHg • IFG:6.1-6.9 mmol/L or IGT
Other criteria	**2 of the following:** • BP≥140/90 mmHg *or treatment* • Dyslipidaemia (TG≥1.7 mmol/L or HDL<0.9(♂)/ 1.0(♀) mmol/L) • Central obesity (BMI≥30 kg/m² or waist-hip-ratio >0.9(♂)/0.85(♀)) • Microalbuminuria (AER ≥20 µg/min or albumin:creatinine ≥30 mg/g)	**2 of the following:** • IFG≥6.1 mmol/L • BP≥140/90 mmHg *or treatment* • Dyslipidaemia (TG>2.0 mmol/L, HDL<1.0 mmol/L *or treatment*) • Central obesity (waist≥90(♂)/ 80(♀) cm)		**2 of the following:** • BP≥130/85 mmHg *or treatment* • TG≥1.7 mmol/L *or treatment* • HDL<1.0(♂)/ <1.3(♀) mmol/L *or treatment* • IFG≥5.6 mmol/L or DM2	**Risk factors** • BMI • Non-Caucasian • Family DM2, CVD • Sedentary lifestyle • CVD, PCOS, NAFLD, gestational DM, acanthosis nigrican

AACE; American Association of Clinical Endocrinologists, ACE; The American College of Endocrinology, AER; albumin excretion rate, BMI; body mass index, BP; blood pressure, DM; diabetes mellitus, EGIR; European Group for the Study of Insulin Resistance, IDF; International Diabetes Federation, IFG; Impaired fasting glucose, IGT; Impaired glucose tolerance, NAFLD; Nonalcoholic fatty liver disease, NCEP-ATP III; US National Cholesterol Education Project Adult Treatment Panel, NHLBI; National heart, lung, and blood institute, OGTT; oral glucose tolerance test, PCOS; polycystic ovary syndrome, TG; triglycerides, WHO; World Health Organization

4.5 Diabetes development in hypertension

4.5.1 Pathophysiology

As part of the metabolic syndrome, hypertension and diabetes mellitus, frequently occur together, and both conditions independently increase the propensity to cardiovascular disease and organ damage, e.g. greater incidence of stroke, coronary heart disease, congestive heart failure, renal failure, peripheral artery disease and cardiovascular mortality[81]. As discussed above insulin resistance may be one pathophysiological mechanism explaining the strong relationship[69]. Endothelial dysfunction and inflammation may be other possible mechanisms e.g. markers of inflammation such as C-reactive protein have been consistently related to incident type 2 diabetes and to increasing blood pressure levels[82-86]. Others have linked low birth weight with adult hypertension and diabetes mellitus[87, 88]. The autonomic nervous system may also be a possible link[89] and will be discussed later. However, as always the sequence of events can be discussed and the question is what comes first?[90]

4.5.2 Consequences

Diabetes mellitus is a major risk factor for cardiovascular disease including microvascular disease and accelerated atherosclerosis with more severe extensive and diffuse lesions compared to those in non-diabetic patients[49, 54]. The risk is added when the patients also have hypertension, and the patients with both diabetes mellitus and hypertension have approximately four times the cardiovascular risk of non-diabetic non-hypertensive subject[49, 50, 54, 81, 91]. The current antihypertensive treatment targets are also lower in diabetic patients (<130/80 mmHg) due to higher risk of cardiovascular endpoints[3].

The risk associated with new-onset diabetes mellitus or incident diabetes mellitus in hypertensives in hypertension trials is not so well characterised. In the up to 16-years follow-up from the observational PIUMA (Progetto Ipertensione Umbria Monitoraggio Ambulatoriale) study, there was a yearly incidence of new-onset diabetes mellitus of 1.9% during antihypertensive treatment, and the patients developing diabetes mellitus during follow-up developed a risk of a subsequent cardiovascular event that approached those with diabetes mellitus at baseline[32, 92]. Hypertension and incident type 2 diabetes increased the risk of coronary heart disease independently, and the combination increased the risk dramatically, particularly in women, in a large Finnish population survey[50]. The coronary heart disease incidence was increased by 23% (1.10-1.37) in men and 2.04 times (1.80-2.30) in women during 21.5-year of follow-up [50]. Results from the MRFIT (Multiple Risk Factor Intervention Trial) trial have shown that the patients developing diabetes mellitus during 18-years of

follow-up had increased total, cardiovascular and coronary heart disease mortality compared to patients without diabetes mellitus[93]. And among the 282 patients developing new-onset diabetes mellitus during 11 years of follow-up in the CHS (Cardiovascular Health Study) study, new-onset diabetes mellitus was associated with an increased risk for all-cause and cardiovascular mortality compared with non-diabetics with a HR of 1.9 (1.4-2.5) and 2.2 (1.4-3.4), respectively[94]. The mortality risk was elevated within 2 years of onset, but surprisingly it did not increase further over time[94]. In a recent published 28-year follow-up study from Sweden there was a yearly incidence of new-onset diabetes of 1.0% in hypertensives, and there was a greater risk for major cardiovascular complications and mortality in subjects who developed new-onset diabetes than in those who did not[95]. However, this was not seen in the same patients at 15 years follow-up[96]. So the results differ, but most likely the risk of developing diabetes is increasing over time.

In a post-hoc analysis from the ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) trial, incident diabetes mellitus was associated with a statistically significant increased risk of coronary heart disease with a HR of 1.64 (1.15-2.32) during the first 2 years of follow-up, but there was no significant increase in any other outcomes in association with the diabetes development[97]. However, the follow-up time was short, fasting glucose measurement was only known for half of the cohort, and information about possible diabetes treatment was not available[97]. Similarly, in the extended follow-up of the SHEP (Systolic Hypertension in the Elderly Program) study, new-onset diabetes was associated with a higher risk of all-cause and cardiovascular mortality in the placebo group, but not in the patients treated with diuretic[98]. However, in these elderly subjects with isolated systolic hypertension and high cardiovascular risk in the short term, the highly favourable prognostic effect of blood pressure reduction may have outweighed the adverse prognostic impact of diabetes development[92, 98]. The patients from the SHEP report were also not closely followed after the randomised part of the trial was stopped and the authors lack information about treatment, blood pressure and diabetes development during later follow-up and the mortality status was assessed from a national database (National Death Index)[98]. From the Swedish follow-up study discussed above we know it can take even longer than 15 years of follow-up to see significant outcome results, and this may also explain why no significant differences were seen in the ALLHAT and the SHEP study follow-up[99].

4.5.3 Differences in diabetes development between antihypertensive treatment regimens

All antihypertensive drugs lower (by definition) blood pressure, and this decline is the best determinant of cardiovascular risk reduction. However, differences between drugs exist with respect of target-organ damage and prevention of cardiovascular events[1]. Recent guidelines recommend five different antihypertensive drugs as first line treatment[3]. However, there are known differences in diabetogenic effect between the regimens[56, 100, 101]. A net-work meta-analysis published in Lancet in 2007 have calculated the odds ratio for new-onset diabetes to be 0.62 (0.51-0.77) for ARB, 0.67 (0.57-0.79) for ACEI, 0.75 (0.63-0.89) for placebo, 0.79 (0.67-0.92) for CCB, and 0.93 (0.78-1.11) for beta-blockers compared with treatment with diuretics (reference drug=1)[56]. In the USA 20 million people are treated with thiazide diuretic and an equal number are on beta-blockers[1]. Based on known diabetogenic risk, this translates into 250 000 cases of new-onset diabetes mellitus associated with these so-called traditional antihypertensive drugs every year[1].

Thiazid diuretics may in high-doses worsen glycaemic control by impairing insulin secretion and decreasing peripheral insulin sensitivity[102]. They may worsen glycaemic control through stimulation of renin secretion and thereby angiotensin II production. Impaired insulin sensitivity has been proposed to be due to increased catecholamine release in response to thiazides[102]. Furthermore thiazide diuretics have a drug- and dose-dependent hypokalaemic effect that may blunt the release of insulin from the pancreas[103, 104], but potassium supplementation and a combination with ACEI or ARB may prevent hypokalaemia.

There is accumulating evidence that beta-blockers increase the likelihood of new-onset diabetes mellitus[42, 105], particularly when combined with thiazide diuretics, as shown in the ASCOT study[106]. Potential diabetogenic mechanisms may include weight gain and unopposed α2 receptor-mediated glycogenolysis, inhibition of pancreatic insulin secretion, alteration in insulin clearance and, probably most important, reduced peripheral blood flow due to increased peripheral vascular resistance[107, 108]. β1-selective blockers with vasodilating action through β2-agonist stimulation or α-blocking activity appear to have minimal detrimental effects on glycaemic control[108].

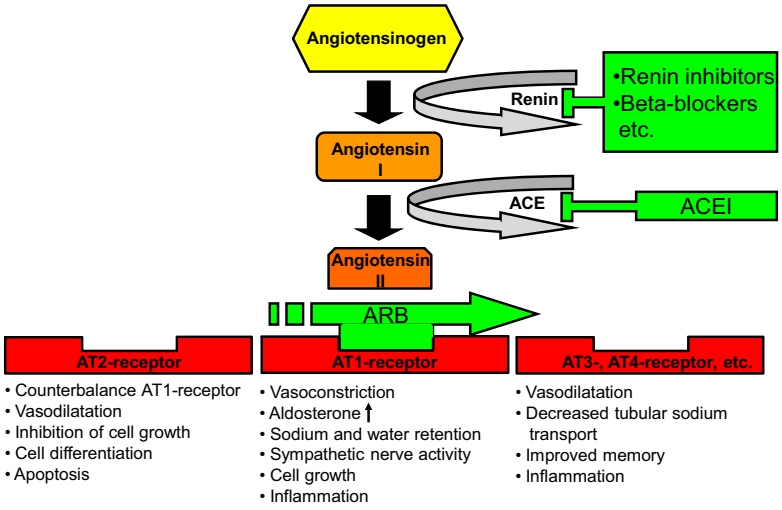
CCBs are considered to be neutral in their effects on glucose homeostasis[56, 109]. Vasodilatation and improved peripheral blood flow may explain the improvement in insulin sensitivity sometimes seen with calcium channel blockade[110]. However, in supra-therapeutic doses CCB are known to inhibit insulin release[111] and some CCBs may activate the SNS[112].

4.6 Renin-angiotensin system (RAS)

4.6.1 Pathophysiology

The RAS is a major neurohormonal regulatory system of cardiovascular and renal function to maintain haemodynamic stability. It plays an important role in the regulation of blood pressure on its own, but it also interacts extensively with other blood pressure control systems including the sympathetic nervous system and the baroreceptor reflexes[113]. RAS is stimulated by e.g. blood loss or excessive loss of sodium and water. The classic activation of RAS occurs from release of renin by the kidney and renin cleaves angiotensinogen into the inactive angiotensin I[114]. The next rate-limiting step is conversion from angiotensin I into angiotensin II by the angiotensin-converting enzyme (ACE) as shown in Figure 1. There are also other activation mechanisms and local production of angiotensin II as well as other angiotensin peptides, which complicate the picture[114, 115]. The final step of the RAS cascade is activation of angiotensin II receptors, and the clinically important ones are type 1 (and 2)[114]. The AT1-receptor belongs to the superfamily of G-protein-coupled receptors and has been localised in most tissues including heart, kidney, vascular smooth-muscle cells, brain, adipocytes, platelets, adrenal glands and placenta[114]. The increased level of angiotensin II increases blood pressure by stimulation of the AT1-receptor by various mechanisms, including vasoconstriction of resistance vessels, aldosterone synthesis and release, renal tubular sodium reabsorption (directly and indirectly through aldosterone), thirst stimulation, release of antidiuretic hormone and enhanced sympathetic outflow from the brain and noradrenaline release[8]. In addition to promote cardiovascular and renal disease, elevated levels of angiotensin II are associated with the development of peripheral insulin resistance[116].

Figure 1. The renin-angiotensin system (RAS) and potential RAS-blockers
(Modified from Aksnes TA et al. Seminars in Cardiology 2006; 12(4):125-135)



4.6.2 Blockers of RAS

The development of pharmacological agents that block RAS specifically have helped to define the contribution of this system to blood pressure control and to the pathogenesis of hypertension and renal failure[114]. Angiotensin II receptor blockade with saralasin in the 1970s lowered blood pressure and improved haemodynamics, but had to be administered intravenously, and in high doses it had some partial angiotensin-II-like effects[114]. Later oral active ACEI and specific blockers of the angiotensin II AT1-receptor were produced. Recently direct oral renin inhibitors have been put on the market[117], and other sympatholytic agents are also in use (e.g. beta-blockers inhibit renin release from the kidney). RAS-blockers have antihypertensive, anti-atherosclerotic, anti-inflammatory, anti-proliferative and oxidative stress lowering effects which protect against cardiovascular, cerebrovascular and renal damage[115].

So far, seven orally active AT1-receptor blockers or ARBs with different pharmacodynamic and pharmacokinetic characteristics are launched in Norway (candesartan, eprosartan, irbesartan, losartan, olmesartan-medoksomil, telmisartan, valsartan). ARBs are

effective antihypertensive drugs. The blood pressure becomes salt-dependent when RAS is blocked, so salt reduction and combination with diuretic increase the antihypertensive effect[114]. ARBs reduce target organ damage like left ventricular hypertrophy, improve haemodynamic indices and reduce cardiovascular and end stage renal disease. Another important advantage is the tolerability of the drug class as the adverse-effect profile is comparable with that seen in placebo groups[118].

4.6.3 Effects of RAS blockers on diabetes development and insulin sensitivity

New-onset diabetes is not a "hard endpoint" like myocardial infarction, stroke and mortality, but has been included as an intermediate endpoint in the recent European guidelines[3]. In the network meta-analysis presented above, the relative odds ratio for developing diabetes was lowest with long-term use of ARBs 0.62 (0.51-0.77) and next lowest with ACEIs 0.67 (0.57-0.79) compared with diuretics[56]. Although a trend, there was no significant difference between RAS blockade compared with placebo or between ARBs and ACEIs[56]. However, these results are predominantly based on data from secondary and post hoc analyses of randomised controlled trials, and there are of course also a possible publication bias due to the fact that older drugs have been longer on the market and more information are known[119]. In the DREAM (Diabetes REduction Assessment with ramipril and rosiglitazone Medication) trial, treatment with RAS blockade was investigated with new-onset diabetes as a primary end point[120], and soon results from the ongoing NAVIGATOR (Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research)[121] and ONTARGET (ONgoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial)/TRANCEND (Telmisartan Randomised AssessmeNt Study in ACE- iNtolerant subjects with cardiovascular Disease)[122] trials will be published and give further knowledge about RAS-blockade and new-onset diabetes. In the DREAM trial treatment with the ACEI ramipril did not reduce the incidence of diabetes mellitus or death, but a significant regression towards normal glucose levels was observed[120]. There is today difficult to conclude whether RAS blockade exerts real anti-diabetogenic action or simply lacks a diabetogenic action possessed by other antihypertensive treatment regimens[3], but more trials are on their way and will hopefully be able to clarify the effect. There are many hypotheses on how RAS-blockade may improve insulin sensitivity and reduce diabetes development that will be discussed more extensively later. However, both haemodynamic effects with better delivery of insulin and glucose to the peripheral skeletal muscle and non-haemodynamic effects including effects on insulin signalling and glucose handling, adipocyte differentiation, hypokalaemia and fibrosis prevention, may be of

importance[100, 123]. The ARB telmisartan has even reported to have partial effect on activity of the peroxisome proliferator-activated receptor–γ (PPAR-γ), a well-known target for insulin-sensitising anti-diabetic drugs[55]. Thus, the effect may involve both improvements of insulin secretion as well as insulin action.

Table 4 shows studies investigating the effect of treatment with ARBs on insulin sensitivity measured with hyperinsulinaemic glucose clamp. The reason for differences between the study results may be due to different study design and duration, different drugs and dosages used, and different patient groups being included in the studies. In spite of some mixed results a possible improvement of insulin sensitivity after treatment with ARBs may be suspected.

Table 4. Studies with ARBs investigating insulin sensitivity measured with glucose clamp

Study	Design	Drugs	Effect ARB (*p*-value)
Akel et al.[124]	8 w parallel treatment of 18 hypertensives	ARB (losartan) and ACEI (enalapril)	NS
Aksnes et al. (Paper II)	8 w double-blind crossover study of 17 mild-moderate hypertensives with other CVD risk factors	ARB (losartan) vs. CCB (amlodipine)	+17%*
Fogari et al.[125]	6 w double-blind crossover study of 25 mild-moderate hypertensives	ARB (losartan) vs. ACEI (lisinopril)	NS
Fogari et al.[126†]	6 w double-blind crossover study in 28 overweight mild-moderate hypertensives	ARB (losartan) vs. ACEI (perindopril)	NS
Fogari et al.[127†]	12 w double-blind parallel-group study of 44 postmenopausal mild-moderate hypertensive women	ARB (losartan) and ACEI (trandopril)	NS
Furuhashi et al.[128‡]	2 w parallel-group study of 16 insulin-resistant mild-moderate hypertensives	ARB (candesartan) and ACEI (temocapril)	+45%*
Higashiura et al.[129‡]	2 w study of 8 mild-moderate hypertensives	ARB (candesartan) vs. placebo	+42%*
Iimura et al.[130]	2 w parallel-group study of 13 mild-moderate hypertensives	ARB (candesartan) and ACEI (delapril)	+42%*
Laakso et al.[131]	12 w double-blind parallel-group study of 20 hyperinsulinaemic hypertensives	ARB (losartan) and BB (metoprolol)	NS
Moan et al.[132]	6 w open-study of severe hypertensives	ARB (losartan) vs. placebo	+30%*
Moan et al.[133]	4 w double-blind crossover study of mild hypertensives	ARB (losartan) vs. placebo	NS
Olsen et al.[134]	1, 2 and 3 year follow-up of 70 hypertensives with ECG-documented LVH (LIFE substudy)	ARB (losartan) and BB (atenolol)	NS
Paolisso et al.[135]	4 w single-blind parallel-group study of 16 insulin-resistant mild-moderate hypertensives	ARB (losartan) and placebo	+30%**
Ura et al.[136]	2 w parallel-group study of 13 hypertensives	ARB (candesartan) and ACEI (delapril)	+42%*

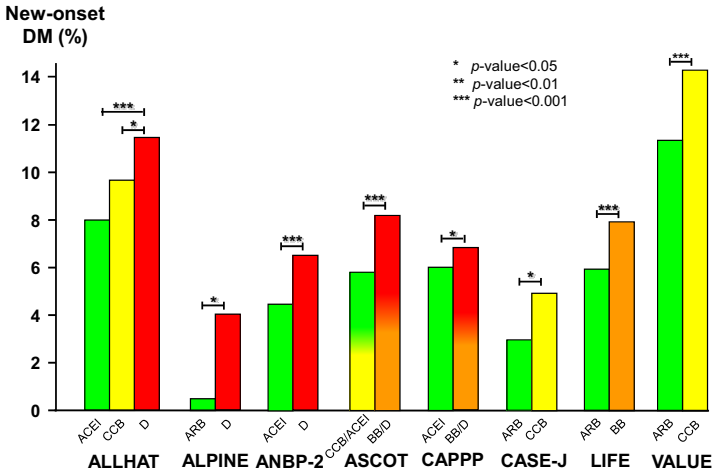
*† and ‡; Partly the same population, *; p-value<0.05, **; p-value<0.01*
(BB; beta-blocker, CVD; cardiovascular disease, LVH; left ventricular hypertrophy, NS; non-significant, w; week)

Large hypertension and heart failure trials have reported impact on diabetes development in favour of RAS-blockade as shown in Figure 2. CCBs have been considered

neutral in case of new-onset diabetes[109]. However, recently ARBs and ACEIs have shown to be associated with significantly less new-onset diabetes than CCBs[56, 137, 138]. The VALUE (The Valsartan Antihypertensive Long-term Use Evaluation) trial was the first opportunity to formally compare the effect of an inhibitor of RAS with a CCB on the development of new-onset diabetes, and the results indicate that the risk for developing diabetes mellitus is either lower or delayed in patients treated with valsartan (ARB) than in those treated with amlodipine (CCB) with a relative risk reduction of 23% (OR 0.77 (0.69-0.87, *p*-value< 0.0001)[137]. Recently also the CASE-J (The Candesartan Antihypertensive Survival Evaluation in Japan Trial) trial has shown a 36% relative risk reduction (HR 0.64 (0.43-0.97), *p*-value=0.033) of new-onset diabetes in patients treated with candesartan (ARB) compared with amlodipine (CCB)[138]. There was also a numerical difference in favour of treatment with ACEIs compared with CCBs in the results from the ALLHAT trail, however the trial design precluded a formal statistical comparison[139]. In the STOP-2 (Swedish Trial in Old Patients with Hypertension-2) study no difference in new-onset diabetes was found[140]. Although a possible favourable effect, it is not establish if RAS blockade reduces or delays the onset of type 2 diabetes mellitus.

Figure 2. New-onset diabetes mellitus in large trials using blockers of RAS[106, 137-139, 141-144]

(ACEI; angiotensin-converting enzyme inhibitor, ARB; angiotensin II-receptor blocker, BB; beta-blocker, CCB; calcium channel blocker, D; diuretics)



4.7 Adipokines

Since 1994 and the discovery of leptin[145], it has been known that adipose tissue is more than a passive storage of fat and energy. Adipose tissue secretes multiple bioactive molecules with local or systemic effects called adipocytokines or adipokines. Adiponectin, leptin, and tumor necrosis factor-alpha (TNF-α) are among the best characterised adipokines and have been linked with insulin resistance and diabetes development. However, the contribution of the various adipokines to the development of insulin resistance is complex and not fully understood. It has also been hypothesised that blockade of RAS promotes the recruitment and differentiation of pre-adipocytes into small insulin-sensitive adipocytes that counteract ectopic deposition of lipids and thereby improves insulin sensitivity[146].

Low plasma levels of the adipokine adiponectin has been associated with obesity and type 2 diabetes mellitus in different ethnic groups, and it has been shown that circulating adiponectin levels correlate better with insulin resistance and hyperinsulinaemia than adiposity and glucose intolerance[147-151]. Adiponectin increases rates of fatty acid oxidation and decreases muscle lipid content which may in part be the underlying mechanism to their

possible insulin sensitising effect[57]. More recently, it has been hypothesised that low adiponectin might be involved in development of hypertension[152, 153]. In Table 5 studies investigating and reporting effects of treatment with ARBs on insulin sensitivity and adiponectin levels are shown, and although some mixed results a trend towards improvement of both insulin sensitivity and adiponectin after ARB treatment may be sensed[128, 154-165]. There are different hypotheses of how RAS-blockade may increase adiponectin levels e.g. RAS-blockade may promote and increase adipogensis and adipocyte differentiation that may result in a greater capacity for adiponecetin production[146]. It has also been shown that RAS blockade may suppress TNF-α synthesis[166], which again suppresses adiponectin expression[167-169]. The effects may also be on gene expression as at least one ARB, telmisartan, has shown to act as a PPAR- γ agonist like the thiazolidinediones[55, 167, 170]. One experimental study has concluded that ARB-induced adiponectin stimulation is most likely to be mediated via PPAR-γ activation involving a post-transcriptional mechanism[171], but this does not explain the positive effect seen of other RAS-blockers with less known PPAR-γ effect[172]. Animal models have also indicated that blockade of angiotensin II-receptor ameliorates adipokine dys-regulation in obese, and that such action is mediated by preventing oxidative stress in obese adipose tissue[173]. In addition to adiponectin's strong association with type 2 diabetes mellitus, a possible association between high adiponectin concentrations and a favourable cardiovascular risk profile has been suggested[174]. However, this association with coronary heart disease is more moderate and requires further investigation[174].

Leptin is polypeptide derived from adipose tissue that promotes weight loss by acting in the hypothalamus to reduce appetite[175]. Results from studies of individuals with leptin deficiency[176] or leptin receptor defects[177], have revealed a critical role of leptin in the normal regulation of appetite and adiposity in humans. The primary biological role of leptin appears to be adaptation to low energy intake rather than to inhibit over-consumption and obesity[170]. Leptin is considered to play a key role in the elevation of sympathetic activity commonly found in obese hypertensive patients[178, 179], presumably by means of increasing caloric expenditure and losing weight[59]. It exerts a direct effect on the kidneys resulting in increased sodium reabsorption and regulates vasomotion[180]. Leptin may decrease muscle lipid content which may in part improve insulin sensitivity[57]. Leptin has also shown pro-atherosclerotic, pro-inflammatory, and pro-thrombotic effects.

The adipokine resistin was discovered in 2001 by screening for genes that were induced during adipocyte differentiation and down-regulated in mature adipocytes treated with thiazolidinediones, and it got its name due to the thinking of this being the linkage

between obesity and diabetes (RESISTance to INsulin)[181]. In mice studies high levels of resistin have shown to correlate with insulin-resistant states, and resistin administration has led to insulin resistance in vivo and in vitro studies[59]. However, there are differences in protein structure between mice resistin and human resistin, and the link between obesity and diabetes in humans has shown to be complicated. Resistin has been shown to directly impair insulin signalling and insulin stimulated glucose uptake in muscle, but has not shown direct effect on altering muscle lipid metabolism.

Plasminogen activator inhibitor type 1 (PAI-1), an inhibitor of fibrinolysis, is another protein related to adipocytes. It has been linked to a variety of biological processes and is secreted by adipocytes, hepatocytes, platelets and vascular smooth muscle and endothelium[182, 183]. Elevated level of PAI-1 may predict future diabetes mellitus and cardiovascular disease in part because elevated levels also reflect visceral obesity and insulin resistance[82, 182, 183]. Weight loss and improvement in insulin sensitivity due to treatment with anti-diabetic drugs have shown significant reduction in circulating PAI-1 levels[183]. In the Framingham Study, PAI-1 had a positive graded relationship with development of type 2 diabetes mellitus, and the association was independent of other risk factors including obesity, homeostasis model assessment for insulin resistance (HOMA-IR), IFG/IGT, triglycerides and inflammation[82].

TNF-α is a pro-inflammatory cytokine and has been suggested to play a key role in insulin resistance in obesity and may contribute to the development of type 2 diabetes mellitus[184]. One possible mechanism may be by impaired insulin signalling and tyrosine kinase activity at the insulin receptor, which is important for the biological activities of insulin[185]. Other mechanisms may be that TNF-α increase release of free fatty acids from adipocytes and reduce adiponectin synthesis[168]. At least one study has shown reduction of TNF-α with ARB treatment[186], and effects through modulation of RAS may be another linkage between TNF-α and insulin resistance.

Table 5. Studies with ARBs reporting effect on insulin sensitivity and adiponectin

Study	Design	Insulin sensitivity	Adiponectin
Aksnes et al.(Paper III)	21 HT, losartan 8w	+	+/-
Benndorf et al.[154]	37 HT, telmisartan 6w	+	+/-
Chujo et al.[155]	28 HT, telmisartan 24w	+/- (+†)	+
de Vinuesa et al.[156]	52 CKD, olmesartan 16w	+	+/-
Furuhashi et al.[128]	16 HT, temocapril/ candesartan 14 d	+	+
Koh et al.[157]	47 HT with hypercholesterolemia, losartan 2m	+	+
Koh et al.[158]	45 HT, candesartan 2m	+	+
Koh et al.[159]	44 HT with hypertriglyceridaemia, candesartan 2m	+	+
Negro et al.[160]	46 HT with IR, irbesartan or telmisartan 6m	+	+
Nielsen et al.[161]	9 men with DM1, losartan 6w	+	+
Park et al.[162]	44 HT with IR and DM, losartan 6m	+	+
Usui et al.[163]	36 HT with DM2, telmisartan 6m	+ ‡	+/-
Yenicesu et al.[164]	21 DM2 with proteinuria, ramipril 4w	+	+
Yilmaz et al.[165]	20 HT with MetS, valsartan 3m	+	+

**** CKD; chronic kidney disease, d; days, DM; diabetes mellitus, HT; hypertensives, IR; insulin-resistant, m; months, MetS; metabolic syndrome, w; weeks, +; positive effect, +/-; neutral effect (non-modified effect)***
† In patients with MetS (n=14). ‡ In patients not taking anti-diabetic drugs (n=14).

4.8 The autonomic nervous system

The nervous system is divided into the somatic nervous system that controls organs under voluntary control (mainly muscles) and the autonomic nervous system (or visceral nervous system) that regulates individual organ function and homeostasis, and for the most part is not subject to voluntary control e.g. regulation of heart rate, respiration and digestion. The autonomic nervous system commands the organs through two antagonistic branches: the sympathetic nervous system, predominant in the active period ("fight, fright, and flight"), whereas the parasympathetic nervous system rules the body in the inactive period ("rest and digest").

4.8.1 The sympathetic nervous system

The sympathetic nervous system is the portion of the autonomic nervous system that enables the body to be prepared for fight or flight. Sympathetic responses include increase in heart rate, blood pressure, and cardiac output and diversion of blood flow away from the skin and splanchnic vessels to the blood vessels supplying skeletal muscle[187]. Efferent sympathetic activity releases noradrenalin and adrenalin from sympathetic nerve endings and from the adrenal medulla, and the cells containing adrenaline and noradrenaline are innervated by separate sympathetic neurons and descending pathways from the hypothalamus[188]. Noradrenaline is the main postganglionic transmitter of the sympathetic nervous system in regulation of the cardiovascular system and sympathetic neural activity evokes and decays slower than the corresponding response to stimulation of the parasympathetic nervous system or vagal activity[189]. This is due to slow noradrenaline release, the slow 2nd messenger system coupling the adrenergic β-receptors and the ion channels, and the slow removal of noradrenaline by cellular re-uptake and urinary excretion[190]. Increased activity of the sympathetic nervous system is thought to play an important role in the pathogenesis and maintenance of essential hypertension, especially in the early phase of hypertension[8, 191, 192]. The specific cause of the increased sympathetic activity in essential hypertension remains largely unknown, although genetic influences are evident and behaviour and lifestyle factors like stress, physical inactivity and obesity are involved[192]. Noradrenaline released from sympathetic nerve endings in the kidney, heart, and blood vessels raises blood pressure by enhancing sodium reabsorption, increasing cardiac output, and increasing peripheral resistance[193]. According to the hypothesis of Julius[68] enhanced sympathetic activity is the primary factor that can be associated with both hypertension and insulin resistance, and possibly obesity. The pressure-induced restriction of the microcirculation limits nutritional flow and thereby impairs glucose uptake in the skeletal muscle[89], which is the major site of insulin resistance[194]. On the other side Landsberg[193] has proposed hyperinsulinaemia as the primary cause of hypertension partly through increased sympathetic activity, and this classic "chicken and egg"-question is still not solved. The adverse effects of sympathetic activation in hypertension are both promotion of atherosclerosis and unfavourable effect on the metabolic profile by development and progression of insulin resistance and dys-lipidaemia[195].

Locally released noradrenaline from sympathetic nerves is likely to increase glucose uptake in skeletal muscle and adipose tissues through β-adrenergic stimulation and through insulin-independent mechanisms[196]. However, noradrenaline does not contribute so much as adrenaline to hepatic glucose production, and in the long term sympathetic activation may

reduce skeletal muscle glucose uptake through microvascular changes[196, 197]. Chronic sympathetic over-activity may produce insulin resistance by receptor mechanisms, decreased capillary density, or by α-receptor-mediated vasoconstriction[89, 198].

Sympathetic activation also increases adipose tissue lipolysis and releases free fatty acids into circulation and thereby producing another mechanism that directly inhibits glucose transport across the cell membrane[59]. There is a direct relationship between the number of sympathetic neural bursts to skeletal muscle tissue and insulin sensitivity assessed by HOMA-IR[199], indicating a linkage between insulin resistance and increased sympathetic activity. The number of sympathetic bursts to skeletal muscle circulation is also greater in diabetic patients as well as in individuals with diabetic parents that still have normal blood glucose[59, 200].

RAS and SNS are linked by a positive feedback relationship[9]. The stimulating effect of sympathetic nerves on renin release from juxtaglomerular cells in the kidney is reciprocated by the sympathostimulation caused by angiotensin II through a variety of peripheral (increase in noradrenaline release from sympathetic nerve terminals, potentiation of the adrenoceptor responsiveness to adrenergic stimuli) and more central (brain and ganglionic influences of angiotensin II) mechanisms[59]. Angiotensin II has shown inhibitory effects on baroreceptor reflex control of heart rate[9, 113]. It facilitates sympathetic and suppresses parasympathetic activity, and RAS blockers may restore the autonomic balance[201, 202].

Dihydropyridine CCBs exert their blood pressure reducing effect through a decrease in peripheral resistance due to arterial vasodilatation and have showed mixed results in relation to SNS[203]. In a study comparing different dihydropyridine CCBs, a significant increase in plasma noradrenaline levels were observed after chronic therapy with amlodipine and felodipine, but not with the lacidipine and manidipine[204]. Sympathoinhibition cannot be obtained by CCBs whose administration may be accompanied by an increase, or at best, no change in sympathetic activity[195]. The possible increase of sympathetic activity by some CCBs, especially triggered by the acute blood pressure reduction, may make them fail to improve metabolic function[195].

Other antihypertensive treatment regimens like diuretics may increase central sympathetic nervous system activity, and alfa-1 adrenergic antagonists may induce a reflectory increase in plasma noradrenaline due to peripheral arterial pressure reduction[205]. As expected beta-blockers and central sympatholytic antihypertensive drugs may reduce SNS activity[205].

SNS in humans may be assessed by heart rate and haemodynamic measurements, noradrenaline measurement in urine and plasma, neurotransmitter release by radiotracer-

derived measurements, microneurographic recordings, heart rate variability (HRV), baroreflex sensitivity (BRS), positron emission tomography (PET) and other imaging techniques[59, 206].

4.8.2 The parasympathetic nervous system

This part of the autonomic nervous system has opposite effects of the sympathetic nervous system and causes a reduction in heart rate and blood pressure, facilitates the digestion and absorption of nutrients, and excretion of waste products from the body[187]**.** The parasympathetic nervous system regulates cardiovascular function through the action of acetylcholine on muscarinic cholinergic receptors. Stimulation of the parasympathetic nervous system by the vagus nerve reduces heart rate, inhibits atrioventricular conduction and reduces myocardial contractility. Due to fast coupling of muscarinic receptors to potassium channels and quick hydrolysis of acetylcholine by acetylcholinesterase, the parasympathetic nervous system allows for beat-by-beat control of heart rate[190].

Parasympathetic cardiovascular control tends to be reduced in patients with essential hypertension. However, this has received much less attention than activation of the sympathetic nervous system, probably due to methodological reasons and due to no direct "parasympathetic" antihypertensive drug[207]. Increased sympathetic activity and decreased parasympathetic activity have been linked not only to hypertension, but also to the associated metabolic abnormalities[198, 208].

Cardiovascular parasympathetic nervous system activity may be studied indirectly through haemodynamic measurements during pharmacologic blockade or assessment of BRS and HRV analyses[207, 209].

5. Aims of the thesis

The aims of the thesis were to investigate insulin resistance and development of diabetes mellitus in hypertensives. The main specific questions addressed can be summarised as follows:

1. Patients with hypertension and high cardiovascular risk may also have a high risk of developing diabetes mellitus. What predicts diabetes development among 15245 high risk hypertensive patients? (Paper I)
2. May additional antihypertensive treatment with AT-1 receptor blockade improve insulin sensitivity measured with the hyperinsulinaemic isoglycaemic glucose clamp compared to treatment with calcium channel blocker alone? (Paper II)
3. We aimed to investigate the influence of AT-1 receptor blockade on circulating adipokines, inflammatory markers and blood viscosity (Paper III)
4. We aimed to investigate if different effects on the sympathetic-parasympathetic balance measured with BRS, HRV and plasma catecholamines may be related to the changes in glucose metabolism after treatment with additional AT-1 receptor blockade compared to calcium channel blocker alone? (Paper IV)
5. May development of diabetes mellitus in hypertensives during antihypertensive treatment affect cardiac outcomes? (Paper V)

6. Material and methods

6.1 The GOAAL study

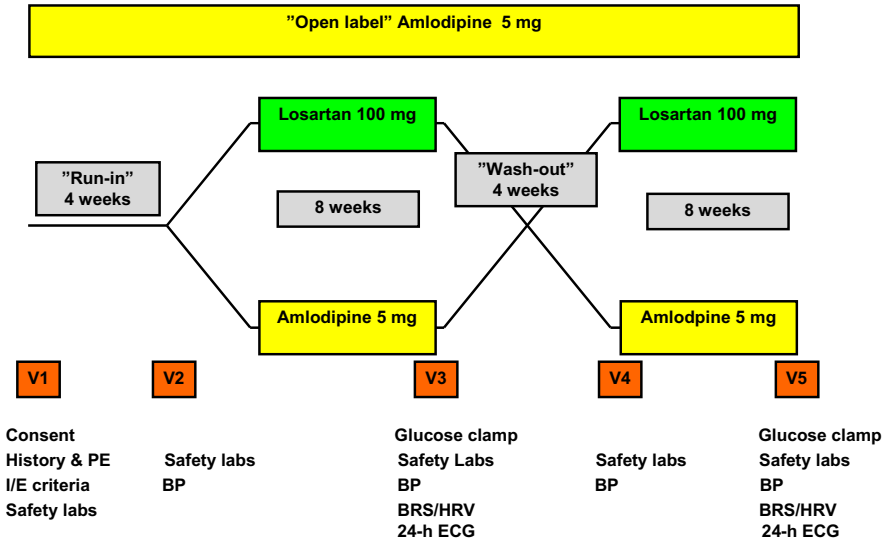
6.1.1 Study design and subjects

The Glucose Optimisation with the Angiotensin II Antagonist Losartan in patients with hypertension and other cardiovascular risk factors (GOAAL) study is a double-blind, randomised crossover study designed to compare the metabolic effects of 10 mg amlodipine and 100 mg losartan + 5 mg amlodipine. After a 4-week open label amlodipine 5 mg run-in period, all hypertensive patients were randomised to additional treatment with either amlodipine 5 mg or losartan 100 mg for 8 weeks (Fig.3). At the end of this 8-week treatment-period, patients underwent blood pressure measurement, blood sampling, a hyperinsulinaemic isoglycaemic glucose clamp and BRS/HRV measurements. Following this was a 4-week wash-out phase where the patients continued open label 5 mg amlodipine, and then they were crossed over to the opposite treatment regimen for another 8 weeks before the final examination with blood pressure measurement, blood sampling, hyperinsulinaemic isoglycaemic glucose clamp and BRS/HRV measurements

The study was approved by the National Committees for Research Ethics in Norway and the Norwegian Medicines Agency. All patients gave verbal and written informed consent to participate before included in the study.

Figure 3. Study design

(Modified after Figure 2 in Paper II)



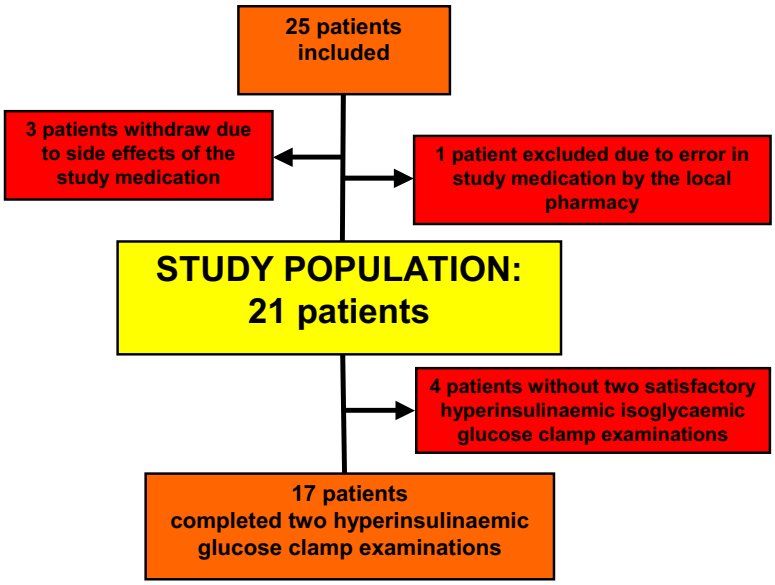
BP; blood pressure, BRS; baroreflex sensitivity, Glucose clamp; hyperinsulinaemic isoglycaemic glucose clamp, HRV; Heart rate variability, I/E; inclusion/exclusion, labs; laboratory tests, PE; physical examination, V1, V2 etc; visit 1, visit 2 etc, 24-h ECG; 24-hour electrocardiography recording

Twenty-five patients with mild to moderate essential hypertension (office diastolic blood pressure 95-110 mmHg and systolic blood pressure <180 mmHg) were recruited from general practitioners in the city of Oslo. The participants were previously untreated (n = 4) for hypertension or treated with monotherapy (n = 21), but not with ACEIs or ARBs. All patients had to have IGT or IFG defined as fasting plasma glucose of 6.1-7.0 mmol/L. The participants also had to have either microalbuminuria (urine albumin excretion rate ≥20µg/min), dys-lipidaemia (HDL-cholesterol <0.9 mmol/L or triglycerides >1.7 mmol/L), BMI >28 kg/m² or an increased waist-to-hip ratio (>0.9 for men and >0.85 for women) according to some of the WHO criteria for metabolic syndrome[24]. At the first visit a clinical examination and laboratory testing with electrolytes, creatinine and thyroid hormones were taken to screen for secondary hypertension.

Due to side effects of the study medication (ankle oedema, headaches, flushing and palpitation), three patients decided to withdraw during the study and are not included in the final analysis. One of the patients who completed the study was excluded from the analyses due to an error at the hospital pharmacy (the patient was given amlodipine 10 mg throughout). Thus, the final study population consisted of 21 subjects (Fig. 4); 11 women and 10 men. The mean age was 58.6 years (range 46-75 years). At inclusion, blood pressure averaged 160 ± 3/96 ± 2 mmHg and heart rate 66 ± 2 beats/min. BMI was 29.2 ± 1.0 kg/m² in the whole study group and the waist-to-hip ratio was 0.92 ± 0.01 in the women and 1.05 ± 0.01 in the men. Four patients (19%) were previously untreated for their hypertension and seven (33%) were previously treated with thiazides, four (19%) with beta-blockers and six (29%) with a CCB. Five (24%) subjects were smokers. Four of the patients did not complete two glucose clamps due to technical problems during the clamp procedure.

Figure 4. Study population

(Modified after Figure 1 in Paper II)



6.1.2 Study endpoints

6.1.2.1 Hyperinsulinaemic isoglycaemic glucose clamp and measurement of insulin sensitivity

A number of methods have been developed for the quantitative measurement of insulin sensitivity, but the hyperinsulinaemic glucose clamp is still considered the "gold standard"[60]. The hyperinsulinaemic isoglycaemic glucose clamp in our study was performed after an overnight fast. Antecubital veins on the right and left arm were cannulated with short Teflon catheters *(Optiva® 2, 18G; Medex Medical Ltd., Haslingden, Great Britain)* and glucose and insulin were infused through one catheter, whereas the other catheter was used for blood sampling. The hyperinsulinaemic isoglycaemic glucose clamp was performed for 120 minutes using a modification of the method described by DeFronzo et al.[60]. The insulin infusion was prepared in a bag with 100 ml of 0.9% saline. To prevent insulin from adhering to the plastics, 4 ml of saline was exchanged with 4 ml of whole blood from the patient, and 30 IE of Insulin Actrapid were then added to the saline + whole blood mixture and shaken well. The mixture was then drawn into a 50 ml syringe and infused at a fixed rate of 0.001 IE/ kg body weight/ min. The fasting blood glucose level was determined as the average of 3 measurements with an Accu-Chek® Sensor *(Roche Diagnostics GmbH, Mannheim, Germany)*. The insulin infusion was kept unchanged during the clamp, and the glucose infusion (200 mg/ml) was started after 5 minutes at a rate of 20 ml/hour and was adjusted every 5 minutes according to the blood glucose level to keep the blood glucose concentration isoglycaemic or at the baseline level. Insulin sensitivity was expressed as the glucose disposal rate (GDR) (mg/kg/min), calculated from the average glucose infusion rate during the last 20 minutes of the 120 minute clamp. This technique for measuring insulin sensitivity has a coefficient of variation of less than 5% in our laboratory[210]. However, the glucose clamp technique is time-consuming and expensive so more easily accessible methods have also been developed.

Fasting insulin per se is a much easier way of measuring insulin sensitivity. However, in patients with pre-diabetes or diabetes the hyperglycaemia is accompanied by inadequate insulin secretion, and the relationship with insulin sensitivity is not so reliable. Therefore different indexes have been developed. The HOMA-IR is calculated in fasting conditions as serum glucose (mmol/L) multiplied by serum insulin (pmol/l) and divided with 135, as described by Matthews et al.[211]. HOMA-IR has shown to correlate well with euglycaemic clamp measures in men and women, young and older adults, and obese and non-obese individuals[211, 212]. HOMA and other indexes are proxies for these more complex measures like the glucose clamp as they do not provide the same depth of physiological information[74].

6.1.2.2 Blood pressure and heart rate

Blood pressure was measured with a mercury sphygmomanometer with adequate cuff size and after 5 minutes rest in sitting position. The pressure was measured at least three times and the values registered were the mean of the two latest measurements. Resting heart rate was measured by pulse palpation for 30 seconds after the blood pressure measurement.

6.1.2.3 Heart rate variability (HRV)

HRV is the oscillation in the interval between consecutive heart beats as well as the oscillations between consecutive instantaneous heart rates[209]. The clinical relevance has been appreciated for more than forty years[209, 213], however, the linkage between reduced variability and cardiac mortality has first been known later[209, 214]. HRV can be measured using time domain and frequency domain analysis of electrocardiographic (ECG) recordings and has emerged as one important non-invasive methods to measure tonic autonomic heart rate control[209, 215].

Time domain variables are calculated from the R-R intervals or normal-to-normal (NN) intervals that is the intervals between two successive normal QRS complexes in the ECG. Thus, the mean R-R interval reflects heart rate, whereas the variable SDNN (standard deviation of the NN interval) is the square root of variance and reflects all cyclic components responsible for variability in the period of recording[209]. Other time domain variables measure high-frequency variations in heart rate, and these include the square root of the mean squared differences of successive NN (RMSSD) and the proportion of interval differences of successive NN intervals greater than 50 ms (PNN50). As the total variance of HRV increases with the length of the recording, the durations used should be standardised e.g. with short-term 5-min recordings and 24-h long-term recordings as suggested by the Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology[209].

Frequency domain variables, derived from power spectral analysis of R-R interval variability, estimate the distribution of power (or variance) as a function of frequency[209]. The power components are very low frequency (VLF; ≤0.04 Hz), low frequency (LF; 0.04-0.15 Hz) and high frequency (HF; 0.15-0.4 Hz) and measurements are usually made in absolute values of power (ms^2), but normalised units (n.u.) of LF and HF may also be measured and represent the relative value of each power component in proportion to the total power minus the VLF component[209]. The normalisation minimises the effect of change in total power and represents the balance of the two branches of the autonomic nervous system[209]. The HF

component reflects efferent vagal or parasympathetic activity, and on the contrary the LF component is thought to reflect both sympathetic and parasympathetic influences[209]. Low HRV and LF have been related to development of hypertension[216], as well as insulin resistance and diabetes mellitus[217, 218]. Both short-term recordings (5 minutes) and long-term recordings (24-hour) may be used in the analyses [209].

We measured HRV using a finger blood pressure monitor (*Finometer®, Finapres Medical System, Amsterdam, The Netherlands)* and a Mingograph 7 recorder *(Siemens Elema, Solna, Sweden)* for 40 minutes during supine rest prior to the clamp procedure. HRV was computed using BeatScope *(BeatScope® Finapres Medical System, Amsterdam, The Netherlands)* and Nevrokard *(Nevrokard® Medistar, Ljubljana, Slovenia)* software program for short-term recordings of 5 minutes for frequency domain methods. The 5 minutes recordings were checked, and the intervals free of missing data and with a minimum of ectopic beats and noise were chosen as recommended[209]. Normalised HF and LF, and LF/HF ratio were analysed. 24-hour Holter recordings were analysed with HRV time domain methods using Novacor Holter software *(Novacor HolterSoft Ultima version 2.3.1, Cedex, France)*. The mean R-R interval, SDNN, and PNN50 were measured. The same person did all the visual checks and manual corrections of individual RR intervals and QRS complex classification.

6.1.2.4 Baroreflex sensitivity (BRS)

In the clinical setting BRS can be assessed by studying either the reflex heart rate response to physiologic activation or deactivation of the baroreceptors obtained by a variety of mechanical or pharmacologic manipulations, or by analysing the spontaneous fluctuations of the arterial pressure in steady-state conditions measured as the ratio between changes in RR interval time and changes in systolic blood pressure (msec/mmHg)[219]. The development of a device for non-invasive measurement of arterial pressure (FINger Arterial PRESsure) has made measurements of BRS more easy accessible[219, 220]. Non-invasively measured "spontaneous BRS" correlates well with results obtained by the pharmacological techniques, and one important advantage is that no external intervention (except cuff application) is required[221, 222]. The cuff pressure oscillations have been found to resemble the intra-arterial pressure wave in most subjects, and changes of blood pressure can be accurately estimated, although the absolute values may be underestimated (or overestimated) in some subjects[220]. BRS measures the dynamic autonomic heart rate control, and cardiac baroreflex response to increase blood pressure is mediated by the parasympathetic nervous system[215, 219]. BRS

increases when there is a shift of the autonomic balance towards an increased parasympathetic dominance[219]. Both BRS and HRV are reduced in high normal blood pressure and hypertensive patients, and even more in hypertensives with hyperinsulinaemia[223-225].

We measured BRS based on the beat-to-beat blood pressure and heart rate recordings performed with the Finometer and the Mingograph 7 as for the HRV analyses, and 5 minutes segments were used for BRS analyses using the Nevrokard software program.

6.1.2.5 Catecholamines

Sympathetic activity can be estimated by measurement of the plasma concentration or urinary excretion of catecholamines and their precursors and metabolites[226]. Measurement of plasma noradrenaline concentration in venous blood represents the most commonly employed index of sympathetic activity in man[226]. Plasma catecholamine concentrations at our laboratory are measured with a validated radioenzymatic technique[227, 228]. Arterial catecholamines may be better than venous catecholamines when comparing hypertensive and normotensive groups[228], as arterial samples reflect the sympathetic tone from heart and kidney better than venous due to skeletal muscle contributes to approximately 50% of peripheral venous noradrenaline[229]. However, due to ethical considerations and discomfort to the patients, we used venous catecholamine. Measurement of plasma noradrenaline represents only a small fraction of the total noradrenaline released from sympathetic nerves and is also dependent on tissue clearance and neuronal re-uptake and does not discriminate between the central or peripheral nature of increased plasma noradrenaline and the regional differentiations[226]. Other measurements of increased sympathetic activity e.g. microneurographic analysis have been considered more optimal[226], but were not used in our study.

Most of the circulating adrenaline is derived from the adrenal medulla[189], and an increase in plasma adrenaline is generally considered to indicate increased adrenaline secretion, although changes in clearance may also modify the concentration[189].

6.1.2.6 Whole blood viscosity (WBV)

Whole blood is a non-Newtonian solid-liquid suspension and whole blood viscosity (WBV) depends on the concentration of cellular elements and the viscosity of plasma. With a rotational rheometer, fluidity of blood can be measured over a range of shear stresses (or shear rates). The liquid is sheared between two surfaces under constant shear stress or shear rate and the resulting shear rate is measured as a response to the applied movement. We measured WBV with a controlled stress rotational rheometer *(CS10, Bohlin Instruments Ltd,*

Lund, Sweden), with a double gap measuring system. The rheological properties of blood in patients with essential hypertension are known to be altered compared to healthy subjects, and WBV is directly correlated to the blood pressure levels[230]. Previous studies from our group have shown that WBV is negatively related to insulin sensitivity[210, 231].

6.1.2.7 Other laboratory analyses

C reactive protein (CRP) got its name due to capacity to bind to the c-polysaccharide of streptococcus pneumoniae and is a known biomarker of cardiovascular disease, and a relationship between CRP and insulin resistance has been shown[232]. The values of high sensitivity CRP (hs-CRP) of < 1, 1-3, and > 3 mg/L correspond with low, moderate, and high cardiovascular risk across a wide group of patient[233]. Measurement of hs-CRP should ideally be performed in a metabolically stable person without obvious inflammatory or infectious conditions and be repeated within two weeks[233], however this was not possible in our study due to the study design.

6.2 The VALUE trial

6.2.1 Study design and subjects

The Valsartan Antihypertensive Long-term Use Evaluation (VALUE) trial is an investigator-designed, prospective, multi-centre, double-blinded, randomised, active-controlled, parallel-group trial. The primary objective was, for the same level of achieved blood pressure, to compare the long-term effects on the incidence of cardiac morbidity and mortality between antihypertensive treatment with the ARB valsartan and the CCB amlodipine. The trial was endpoint driven and the patients were followed for 4-6 years with regular visits, and upward-titration of medication was implemented in five steps to reach a goal blood pressure below 140/90 mmHg. The trial included 15245 patients older than 50 years who were treated (92%) or untreated for essential hypertension at baseline[234]. Previously untreated patients were included if they had a mean sitting systolic blood pressure between 160 and 210 mmHg (inclusive) and a mean sitting diastolic blood pressure below 115 mmHg, or a mean sitting diastolic blood pressure between 95 and 115 mmHg (inclusive) and a mean sitting systolic blood pressure below 210 mmHg[235]. For patients already on antihypertensive treatment the mean sitting blood pressure should not exceed 210 mmHg systolic and 115 mmHg diastolic, but there was no lower limit. Additional inclusion criteria were the presence of predefined combinations of cardiovascular risk factors and/or disease factors according to an algorithm based on age and gender[235]. The qualifying risk factors included diabetes mellitus, cigarette

smoking, hypercholesterolemia, proteinuria, serum creatinine >150 μmol/l and left ventricular hypertrophy (LVH) without strain on ECG using Cornell[236] or Sokolow-Lyon criteria[237]. The qualifying disease factors included documented history of myocardial infarction or significant coronary heart disease (e.g. documented on arteriogram), peripheral vascular disease, cerebral stroke or transient ischemic attack, or the presence of LVH with strain on ECG. Women aged 50-59 years had to have at least one disease factor and two risk factors, while men in the same age-group only had to have one disease factor or three risk factors to enter into the trial. For men and women 60-69 years at least two risk factors or one disease factor were required, and for patients above the age of 70 years only one risk factor or one disease factor were required for randomisation.

6.2.2 Study endpoints

6.2.2.1 New-onset diabetes mellitus

We wanted to investigate patients patients that developed diabetes mellitus during the VALUE trial. All patients who at entry were diagnosed as diabetics, received anti-diabetic agents, or had abnormal glucose levels were excluded in the analysis of new-onset diabetes mellitus. To detect new-onset diabetes mellitus in the VALUE trial three different criteria were used, but patients could only be counted once. The criteria were:

1. Diabetes mellitus reported by the investigators as an adverse event during the trial. (The investigators were strongly encouraged to use the WHO 1999 criteria[24])
2. Information about new use of oral anti-diabetic drug or insulin in study reports. This database contained a detailed directory of drugs by both generic and trade names in all participating countries.
3. Fasting glucose concentration ≥7.0 mmol/L in a venous blood sample drawn at study end and analysed in a central laboratory.

Diabetes mellitus was at baseline[234] defined by the WHO 1985 criteria (fasting glucose ≥7.8 mmol/L on at least two separate occasions)[238]. In 1999, during the course of the study, WHO changed the definition of diabetes mellitus to a fasting blood glucose of ≥7.0 mmol/L and/or blood glucose ≥11.1 mmol/L two hours after oral intake of 75 g glucose[24]**.** During the blinded phase of the trial, the working classification of new-onset diabetes mellitus was therefore redefined to adhere with the WHO 1999 criteria[24], and this protocol was pre-specified in a

study newsletter. Consequently new-onset diabetes mellitus in our manuscripts is defined as fasting blood glucose of ≥7.0 mmol/L during the trial in patients without diabetes mellitus or with blood glucose <7.0 mmol/L at entry. The new definition increased the number of diabetics at entry of the VALUE trial by 427, and these patients are also excluded from the analysis of new-onset diabetes mellitus.

According to these criteria, we have divided the 15245 patients in the VALUE trial into three pre-specified groups; patients with diabetes at baseline, patients that developed diabetes during the average 4.2 years of the trial and patients without diabetes both at baseline and at the end of the trial. 5250 patients were diabetic at baseline, and 1298 of the initial 9995 non-diabetic patients developed diabetes mellitus during the average 4.2 years of the VALUE trial.

7. Summary of results

7.1 Paper I

The risk of developing diabetes mellitus among the non-diabetic VALUE trial population of hypertensive patients with high risk of cardiovascular disease was investigated. Easily accessible baseline clinical characteristics (glucose, BMI, non-Caucasian race, low age, heart rate and history of coronary heart disease (CHD)) predict patients at risk of developing diabetes mellitus. Baseline blood glucose and BMI were by far the most important baseline predictors of new-onset diabetes mellitus in the VALUE trial population.

7.2 Paper II

Additional treatment with ARB improved insulin sensitivity (GDR) measured with hyperinsulinaemic isoglycaemic glucose clamp, compared with treatment with CCB alone despite similar blood pressure reduction. The GDR was significantly higher after treatment with losartan 100 mg + amlodipine 5 mg compared to amlodipine 10 mg (4.9 ± 0.4 vs. 4.2 ± 0.5 mg/kg/min, *p*-value=0.039) in hypertensives with other cardiovascular risk factors. Thus our data suggest that AT-1 receptor blockade with losartan improves glucose metabolism beneficially through mechanisms at the cellular level, beyond what can be expected by the vascular vasodilating effects and blood pressure reduction alone.

7.3 Paper III

No significant differences in adipokines, viscosity, inflammatory and fibrinolytic markers were found after treatment with losartan 100 mg + amlodipine 5 mg compared to treatment with amlodipine 10 mg. The difference in insulin sensitivity previously found after additional treatment with ARB compared with CCB alone is most likely caused by other mechanisms.

7.4 Paper IV

Plasma noradrenaline was significantly lower after additional treatment with ARB, 304 ± 29 pg/ml after treatment with losartan 100 mg + amlodipine 5 mg compared to 373 ± 43 pg/mL after treatment with amlodipine 10 mg (*p*-value=0.022). HRV, BRS or plasma adrenaline did not differ significantly between the two treatment regimens. The reduction seen in plasma noradrenaline may indicate a potential beneficial peripheral mechanism on the noradrenaline turnover. We think that improvement of insulin sensitivity

and possible reduction in diabetes development by blockers of RAS may be related to the decreased plasma noradrenaline and potential sympatholytic effects.

7.5 Paper V

In the high-risk hypertensive VALUE population, patients with diabetes mellitus at baseline had higher cardiac morbidity and mortality than patients without diabetes. Also patients that developed diabetes during the average follow-up of 4.2 years of the trial had higher cardiac morbidity. This indicates that these patients who develop diabetes during antihypertensive treatment have cardiac morbidity intermediate between diabetics and never diabetics, and that it is of importance to find these patients at risk of diabetes development and optimise life style and medical treatment.

8. Discussion

8.1 Methodological considerations

8.1.1 Study subjects and methods

8.1.1.1 The GOAAL study

The acronym GOAAL was not used in the papers because we did not want to emphasise on a special generic drug, but on the ARB drug class as a whole. Patients in the GOAAL study were recruited due to being at special risk of developing diabetes mellitus. In a previous study from our group we found that open treatment with the ARB losartan for 6 weeks in patients with severe hypertension (untreated diastolic blood pressure (BP) ≥115 mmHg or treated >95 mmHg) improved insulin sensitivity and reduced plasma noradrenaline significantly[132]. However, in a follow-up randomised double-blind placebo-controlled crossover study, treatment of mildly hypertensive patients (office BP >140/95 mmHg or home diastolic BP > 90 mmHg) with losartan for 4 weeks had neutral effect on insulin sensitivity[133]. Although, not an endpoint in the study, the authors found improvement of insulin sensitivity in the most insulin-resistant patients[133]. These results may be due to "regression towards the mean", however we decided to investigate the effect of ARB treatment in patients at special risk of being insulin-resistant. Patients with glycaemic disarrangement like IGT or IFG are at risk of developing diabetes mellitus. Post-load hyperglycaemia reflects the acute increase in blood glucose after a glucose load and peripheral insulin sensitivity, whereas fasting blood glucose shows the glucose concentration after an overnight fast and reflects mostly hepatic glucose production. Other inclusion criteria in the GOAAL study were obesity and dys-lipidaemia, criteria often included in metabolic syndrome and known risk factors for diabetes development[24].

We used a crossover design with 8 weeks treatment-periods due to knowing the full antihypertensive effect of losartan is evident first after 6-8 weeks[133]. Since all the patients were moderate hypertensives and required antihypertensive medication, a placebo-controlled study was not an option. We used a CCB, amlodipine, as the comparator due to also being a vasodilator and to investigate if an ARB could have an effect beyond the vasodilating effect of a CCB. It would have been better to compare a CCB with ARB alone, but as all the patients were hypertensives in need of antihypertensive treatment, we did not find it ethical to take them off medication for the "run-in" and "wash-out" periods and the patients were therefore treated with open-label amlodipine 5 mg throughout the study.

Insulin sensitivity was measured using the hyperinsulinaemic glucose clamp. It's considered to be the "gold standard" due to the direct measurement of the insulin effect to promote glucose utilisation[60]. In the early studies of insulin sensitivity from our group the blood sampling arm was warmed with a heating sleeve or cuff in order to arterialise venous blood for the estimation of arterial blood glucose (i.e. the stimulus to insulin secretion)[133]. This manoeuvre gives higher blood glucose values and may affect the estimated glucose disposal, but it may also induce haemodynamic effects like systemic vasodilatation and reflex tachycardia that might have an effect on glucose metabolism, so in the latest studies from our group we have not used the heating cuff[239]. There is a lack of consensus regarding the optimal length of the glucose clamp, but 120 minutes clamp procedure is often used[60, 61]. The time to insulin action is slowed in obesity and non-insulin dependent diabetes mellitus[240], and a shorter and less time-consuming clamp examination of our patients with obesity and risk of diabetes mellitus development may have influenced the results due to not reaching steady state before ending the clamp procedure and was not an option.

Oral glucose tolerance test was not included in our study in order to minimise the strain on the recruited volunteers. If we had included an oral glucose tolerance test, the patients had to meet at the hospital on two separate days for each examination and we therefore chose to focus on the "gold standard", the glucose clamp, for examination of insulin sensitivity.

HOMA-IR analysis in a short- term study is questionable, but it has been included for the completeness of the results. We measured insulin in pmol/l and have therefore calculated HOMA as serum glucose (mmol/L) multiplied by serum insulin (pmol/l) and divided with 135[241], compared to dividing with 22.5 when insulin is measured in mU/L. This means that the values of HOMA-IR in our study can be compared with the HOMA-IR used in other studies measuring serum insulin in mU/L.

Adipokines in the GOAAL study was investigated in venous blood samples, and possible differences in tissue (adipose and skeletal muscle tissue) in our patients cannot be ruled out.

8.1.1.2 The VALUE trial

The VALUE trial is a large multicentre trial including patients from more than 30 countries, and large trials have advantages and disadvantages. In trials like this there is a trade-off between number of variables included and making busy investigators able to cope with the registration schemes. National and local differences in investigation and treatment strategies

are also challenging. The patients were included according to the presence of predefined combinations of cardiovascular risk and disease factors, and this is due to the fact that most trials need high-risk patients to get enough endpoints to prove significance, but of course this may cause some limitations in the possibility to generalise.

The primary endpoint in the VALUE trial was cardiac morbidity and mortality. However, new-onset diabetes was pre-specified as an endpoint in a study newsletter during the trial. The definition of diabetes was changed during the blinded phase of the trial to adhere with the WHO 1999 criteria[24], and the investigators were encouraged to use the WHO 1999 criteria when reporting diabetes mellitus and adverse events. We used three different databases to investigate new-onset diabetes in the VALUE trial (adverse events, antidiabetic drugs and a blood sample at study end). The patient could qualify with one, two or three criteria, but could only count once in the final database. By using three different databases the results were made more robust, however, in some patients the diagnosis were made on the basis of only one single blood sample at study end. On the contrary the baseline diagnosis of diabetes may also be on the basis of only one glucose value, and as known from the ELSA (European Lacidipine Study on Atherosclerosis) study a substantial proportion (42%) of the initial baseline diabetes mellitus diagnosis could no longer be confirmed at study end after average 3.8 years using the same criteria[242]. This indicates the vulnerability in the analysis using only one blood sample for the diabetes diagnosis, however this is a trade-off in large trials.

There was a relatively high incidence of new-onset diabetes (3.1% per year) in the VALUE trial compared with other hypertension trials like ALPINE (2.3% per year)[141], ALLHAT (2.6% per year)[139], CAPPP (1.1% per year)[143], LIFE (1.5% per year)[144], and NORDIL (1.0% per year)[243]. However, this can be related to different diagnosis criteria of new-onset diabetes, using the WHO 1985[238] or the more strict WHO 1999[24] criteria for diabetes, and the high-risk population included in the VALUE trial.

Investigation of cardiac outcomes in the patients with new-onset diabetes mellitus was a prespecified analysis in this prospective randomised trial, however, the analyses were by nature retrospective and analysed after the trial was ended. We included all clinical events during the trial period for the patients in the new-onset diabetes group; also events happened before the actual diagnosis of diabetes. This can be discussed, but was done due to knowing that the risk of cardiovascular disease is increased long before the clinical diagnosis of diabetes[35].

8.1.2 Statistics

8.1.2.1 The GOAAL study

Statistical analyses in the GOAAL study were performed using SPSS version for Windows *(SPSS Inc., Chicago, Illinois, USA)*. The distribution of each data sample was tested for normality, and initial non-normally distributed variables were tested again for normality after natural logarithmic transformation. Based on this consideration, either parametric or non-parametric statistical tests were used. Continuous variables were examined for statistical significance using paired sample t-test. Null hypotheses were rejected if the *p*-values were below 0.05. Data were given as mean values and their standard errors (SEM).

In a crossover study the same group of patients are given both treatment regimens in sequence, and randomisation is used to determine the order in which the treatments are received. This means that the comparison between the treatments is "within-subjects" rather than "between-subjects", and the sample size needed is smaller[244]. However, carry-over effects may appear with the crossover design meaning that the results obtained during the second treatment period are affected by what happened in the first period[244]. We therefore used a wash-out period of 4 weeks between the treatment periods. This may minimise carry-over effects, but possible carry-over effects must still be considered in the results. One way to assess carry-over effects from the initial treatment period to the next is to compare the percent changes from baseline to the respective first and second treatment period. However, in our study we did not have insulin sensitivity (and most of the other endpoint parameters) at baseline. This design was chosen to minimise the stress on the patients and because differences in blood pressure must have been considered if baseline values were used, and would have made the analyses of baseline variables difficult. The results from baseline or visit 1 are not homogenous as the patients then were untreated or treated with different anti-hypertensives. However, baseline variables measured have been included in papers to better describe the study-population.

Nine of the 17 patients who successfully completed two glucose clamp examinations were treated with amlodipine in their first crossover period, and GDR on amlodipine 10 mg treatment was the same for the patients randomised to this treatment in their first crossover period (4.2 ± 0.8 mg/kg/min, n = 9) and those randomised to this in the last crossover period (4.2 ± 0.6 mg/kg/min, n = 8). The GDR on losartan 100 mg + amlodipine 5 mg was 4.5 ± 0.4 mg/kg/min in the patients given the losartan treatment regimen in the first crossover period, and 5.2 ± 0.6 mg/kg/min in the patients given the losartan treatment regimen in the second

crossover period. This indicates an effect of additional ARB treatment and no carry-over effect.

There may also be a systematic difference between the two treatment periods in a crossover design e.g. observations in the second period may be lower (or higher) than those in the first period, regardless of treatment[244]. No such "period effect" was seen in our study.

One other disadvantage of the crossover design is that unsuccessful analyses or withdrawal, e.g. due to side-effects, exclude the patients from the analyses. This decreases the sample size and the statistical power, and unfortunately we lost four patients in the analysis of GDR due to technical problems during one of the two clamp procedures. The sample size of 22 patients was made on calculations of expected difference in insulin sensitivity between the two treatment regimens based on previous studies[133]. And the sample size was not calculated based on secondary endpoints like adipokines, inflammatory markers, HRV and BRS. These analyses may be vulnerable for type II errors meaning that non-significant results may in fact be due to a too small sample size and lack of power, and not due to a true null hypothesis. In our analyses of adipokines and inflammatory variables there were consistent results with no difference between the treatment regimens (except maybe for adiponectin) so we concluded that there was no significant difference in these variables (Paper III). The sample size in our study was also comparable to previous studies on adipokines. The lack of significant difference in the HRV and BRS analyses may also be due to a type II error as retrospective power calculations according to the standard deviations in our HRV analyses estimated that we would have needed a sample of 30-200 patients to find significant differences in some of the different HRV analyses (Paper IV).

8.1.2.2 The VALUE trial

In the VALUE trial Statistical Analysis System *(SAS Inc. Cary, North Carolina, USA)* was used for all statistical analyses, and all tests were 2-sided, and the significance level was set at 5%. Data are expressed as mean and their standard deviation (SD) for continuous variables and categorical variables are presented as frequencies and percent (%).

Both univariate and multivariate logistic regression models were used to evaluate baseline demographic, risk and disease factors, baseline laboratory variables and prior antihypertensive medication that significantly predicted diabetes mellitus development (Paper I). Twenty-five potential baseline predictors of new-onset diabetes mellitus were identified in the trial database, and univariate logistic regression analyses were used to identity predictors with a significant *p*-value of below 0.05 and to calculate odds ratios for diabetes development.

Multivariate stepwise logistic regression analyses were used to further define significant baseline predictors in four different models. A stricter *p*-value was used in the multivariate analyses (*p*-value <0.001) than in the univariate analyses to get a simple model with the most important predictors of diabetes development. The results are presented as Chi-Square (χ^2) with correspondant odds ratios. An odds ratio > 1 indicates an increased risk of diabetes mellitus development, while an odds ratio <1.0 indicates reduced risk. We used the odds ratios from the final multivariate model and compared them to the univariate model to make sure that the results were consistent. For example the odds ratio was 2.179 for baseline glucose in the univariate analysis vs. 2.106 in the final multivariate model, and this indicates consistency.

To provide additional validation of the results from the multivariate stepwise model building, the patients with new-onset diabetes mellitus and those without were randomly split in two; a learning sample of 40% (3999) and a validation sample of 60% (5996) of the patients. The model building using multivariate stepwise logistic regression was then repeated on the learning sample with a significance criterion of *p*-value <0.05, and the identified model was then checked on the validation sample with a criterion of *p*-value <0.05.

A Cox regression model for endpoints was used when analysing cardiac endpoints in the patients with baseline diabetes, new-onset diabetes and without diabetes (Paper V). The VALUE trial was an event driven trial, and patients in the trial have different numbers of years of follow-up. We were missing the exact dates for diagnosing new-onset diabetes (e.g. debut of adverse events or anti-diabetic drugs were reported without dates in 87 patients or 6.7% of the total 1298 patients with new-onset diabetes) and in the database these patients with new-onset diabetes are assigned to have new-onset diabetes at randomisation date.

In the primary analyses of cardiovascular endpoints we adjusted for pre-defined covariates (age, diabetes status, LVH, CHD and randomised study treatment). However, other known covariates were included in a secondary Cox regression model (baseline and in trial use of aspirin, statins, beta-blockers, diuretics, diuretics and beta-blocker combination, blood pressure, heart rate and sex). Pair-wise comparison between patients without diabetes and patients with diabetes at baseline as well as patients without diabetes and patients with new-onset diabetes were performed with corresponding HRs, and the patients without diabetes mellitus both at baseline and at the end of the trial (never diabetes) were used as comparator. Event rates over time by the three groups were also presented as Kaplan-Meier curves.

Given the overall large number of patients studied in the VALUE trial some results, although statistically significant, may not be clinically relevant. There may also be type I

errors meaning that the null hypothesis is rejected, i.e. a significant test result is demonstrated, when the null hypothesis in fact is true. However, as the results are consistent throughout different models of univariate and multivariate logistic regression analyses and after additional validation, the results are most likely to be reliable and robust (Paper I). As always, one should be cautious in interpreting results from regression analysis, which only demonstrates an association between the dependent and independent variables and does not necessarily imply a causal effect.

8.2 General discussion

8.2.1 Detection of hypertensives at high-risk for diabetes development

Higher-than-optimum blood glucose is a leading cause of cardiovascular morbidity and mortality worldwide[245], and it is of great importance to detect patients at risk of developing diabetes mellitus, as many remain unaware of their disease, and complications may develop before the actual diagnosis[35, 43]. Instead of a general screening for diabetes mellitus with blood glucose measurement of all patients, identifying subgroups with special risk may be cost-efficient. In fact a survey from Great Britain has shown that screening for diabetes mellitus in general practice by measuring fasting blood glucose has a very low yield in low risk populations[246]. However, screening in high-risk populations may be cost-effective. Patients with assumed metabolic abnormalities, including those who are obese, hypertensive or have prevalent cardiovascular disease or a family history of diabetes may be of interest for further testing and different risk scores have been developed for these purposes e.g. Diabetes Risk Score[247] (or FINDRISC[248]), Diabetes Risk Test[249].

In the VALUE trial including high risk hypertensive patients, investigation of 25 baseline predictors showed that six easily accessible baseline predictors (glucose, BMI, non-Caucasian race, low age, heart rate and history of CHD) were significant predictors of new-onset diabetes mellitus in a multivariate model including all patients and excluding randomised treatment (Paper I). The randomised study treatment with amlodipine or valsartan was not included in the final chosen model due to making the model more general and better to use in other settings.

Glucose at baseline was by far the most important risk factor for new-onset diabetes mellitus in all analyses and models used in our study, and this was not unexpectedly due to previous results[33, 34]. BMI was the next most important predictor of new-onset diabetes mellitus in all our analyses. Obesity and abdominal fat distribution are well-known risk factors for type 2 diabetes mellitus. The natural history of diabetes mellitus development may

be exaggerated by obesity because elevated levels of free fatty acids may both directly impair peripheral glucose utilisation and increase hepatic gluconeogenesis[250], and indirectly affect hepatic auto-regulation through altering hypothalamic sensing[251]. Altered secretion of adipokines from adipocytes or macrophages in fat tissue may be another mechanism involved in the dys-regulation between fatty tissue and glucose metabolism. Waist circumference may predict diabetes mellitus better than BMI, as BMI does not differ between fat and muscles and may overestimate obesity especially in young men[37]. However, BMI was the measurement of obesity in the VALUE trial and was a highly significant predictor of new-onset diabetes mellitus in both univariate and multivariate analyses.

The prevalence of diabetes mellitus is increasing with age[252], but in our VALUE trial population increasing age at baseline was associated with a lower risk of developing diabetes mellitus during the trial-period. We assume this is caused by selection, and the inclusion criteria inasmuch as only high-risk hypertensive patients above the age of 50 could qualify for the study, i.e. high-risk elderly hypertensives may by being survivors of long-standing hypertension without diabetes mellitus development, gradually lose the metabolic ability of the disease, and these elderly hypertensives may survive because they do not have the same underlying risk of metabolic disease as the younger hypertensives. Partially, this was also seen in the LIFE (Losartan Intervention For Endpoint) study[144, 253] and in the recently published results from the ASCOT-BPLA (Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm) study[254]. Epidemiological data included in the DECODE (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) study have shown that fasting and post-load glucose values do not identify the same patients and are influenced differently by the aging process[252]. IFG prevalence tends to plateau in middle age, whereas the prevalence of IGT continues to increase also into older age[25, 252]. In our study we mainly defined diabetes mellitus according to fasting glucose values, which may be another explanation for the age-findings in VALUE and other trials[144, 253, 254].

There are known differences in diabetic risk between races, and African Americans are known to have an elevated risk of diabetes mellitus compared with Caucasian[29]. In the VALUE trial most of the patients were Caucasian (91.1%), and patients that were non-Caucasian (3.2% black, 3.4% oriental, 2.3% other races) developed more diabetes mellitus. Baseline heart rate was a significant predictor of new-onset diabetes mellitus in all our analyses except the multivariate analysis in model 4 (patients treated with amlodipine). These results suggest a possible sympathetic drive as an underlying pathophysiological mechanism for diabetes development, and a possible sympathetic effect of amlodipine may have

diminished the effect of heart rate as a predictor in the amlodipine-treated patients[89, 195]. A history of CHD was a significant predictor in both the univariate and multivariate model including all patients, but a previous history of cerebrovascular disease or peripheral arterial occlusive disease were not significant predictors in any of the analyses. This may be due to fewer patients included with these diseases or a different relationship between different risk factors and different cardiovascular diseases. Randomised study treatment was a highly significant predictor of new-onset diabetes mellitus, and treatment with the ARB valsartan reduced new-onset diabetes mellitus by an absolute 3% and a relative 23% compared with amlodipine in the VALUE trial[137]. However, we decided not to include study treatment in our final model due to making the model more general.

In the LIFE study, 562 of the 7998 non-diabetic hypertensive patients with ECG-documented LVH developed diabetes mellitus during the average follow-up of 4.8 years[144]. LIFE was the first study that made use of the ability to analyse the appearance of new-onset diabetes mellitus in patients with different levels of risk and significant predictors of diabetes development from multivariate analyses were non-fasting glucose, BMI, HDL-cholesterol, systolic BP and prior use of antihypertensive drugs[144]. HDL-cholesterol was not studied as a predictor in the VALUE trial, and systolic BP was not a significant predictor in our univariate analyses. This is possibly due to the roll-over design in the VALUE trial which did not include a wash-out period without antihypertensive treatment, and thus lack “true” baseline BP values[234, 235].

The recently published results from the ASCOT-BPLA showed that 1366 of the 14120 patients "at risk" of developing diabetes subsequently developed diabetes during the median follow-up of 5.5 years[254]. Increasing fasting plasma glucose, BMI, serum triglyceride and systolic BP were associated with diabetes development, while randomised treatment with the CCB amlodipine ± ACEI perindopril, high HDL-cholesterol, alcohol use and age were found to be protective factors against diabetes development[254]. The most powerful predictor of new-onset diabetes mellitus in the ASCOT-BPLA was as in the VALUE trial, glucose level which increased the risk of diabetes development 5.8-times (5.23-6.43) for each mmol/L rise of glucose above 5 mmol/L[254].

In the CAPPP (Captopril Prevention Project) study glucose, BMI, haemoglobin, age, interaction between systolic BP and newly diagnosed hypertension, total-cholesterol and prior antihypertensive treatment were significant risk factors in a multivariate analysis[143, 255]. The CAPPP study included low-risk patients from only Sweden and Finland indicating a less general population than the VALUE trial.

In a 28-year follow-up of 754 hypertensive patients from Sweden, 148 patients (20.4%) developed diabetes and BMI, triglycerides and treatment with beta-blockers were independently and positively related to the diabetes development in a multivariate Cox regression analysis[95].

Information about family history of diabetes mellitus and physical activity, diet, alcohol use, gestational diabetes, waist-hip-ratio, insulin, triglyceride and HDL-cholesterol levels, all well-known and strong risk factors for diabetes mellitus development, are not included in the VALUE database. On the other hand the baseline predictors found in our study can easily be evaluated and used by physicians in treating patients in daily practice. The VALUE trial included mainly Caucasian patients (91.1%), and this limits the possibility to generalise. The age- and gender-algorithm used for recruitment also limits the precision of using these two variables as completely reliable predictors. The investigators were urged to use the WHO 1999 definition of diabetes mellitus[24], but we can not rule out some misclassification by the investigators. Large-scale randomised clinical trials are not designed to investigate pathophysiological issues and can only suggest possible mechanisms behind development of diabetes mellitus, but may generate hypothesis to be investigated in other studies. The proper validation of results like these is either by evaluating the risk factors in a prospective non-selected population based cohort or applying the results on other similar trial data (which is expensive and complicated from an administrative point of view due to different investigators and different pharmaceutical companies sponsoring other large trials), or to split the data into two halves using one for the model and the other for validation, which we have done in this context.

However, the predictors for diabetes development found in the VALUE trial population might help in identifying patients at risk, and within the context of limited health care resources it is sensible to focus initially on those at the highest risk of developing diabetes, and these patients should receive the most effective investigations and interventions available.

8.2.2 Possible mechanisms for improved insulin sensitivity and reduction of new-onset diabetes mellitus seen with blockers of RAS

In the GOAAL study we found that in hypertensive patients treated with additional ARB losartan significantly improved insulin sensitivity as assessed by the glucose clamp technique, and there was also a trend towards lower values of HOMA-IR (Paper II). Insulin-mediated glucose uptake during the hyperinsulinaemic isoglycaemic glucose clamp occurs mainly in

skeletal muscle and is dependent on muscular blood flow[60]. Thus, an increase in blood flow induced by vasodilating drugs would be expected to increase glucose uptake. The observed effect in the GOAAL study was beyond the vascular effects and blood pressure reduction achieved by maximal calcium channel blockade with amlodipine as the blood pressure reduction was similar during the two treatment periods. This indicates a possible non-haemodynamic effect of the AT1-receptor blocker explaining the improvement in insulin sensitivity.

One hypothesis is that blockade of RAS may reduce the risk of diabetes development by stimulating proliferation and differentiation of pre-adipocytes into small insulin-sensitive adipocytes[146]. This prevents ectopic fat storage e.g. in liver, skeletal muscle, and pancreas, which is associated with insulin resistance. Skeletal muscle is responsible for most of the insulin stimulated glucose disposal and takes up significant quantities of plasma fatty acids for energy production (oxidation) or for storage in intracellular lipids[57]. Thus an elevation in circulating fatty acids in obese humans results in increased uptake and excess deposition of lipids within the muscle cell and aggravates insulin resistance. Reduced plasma adiponectin is strongly and independently associated with insulin resistance and type 2 diabetes development[57, 148]. We also found that adiponectin concentrations in our patients correlated well with insulin sensitivity as measured with the hyperinsulinaemic isoglycaemic glucose clamp, and this was the only investigated adipokine with a significant correlation with insulin sensitivity after both treatment periods. However, no significant differences were found in adipokines between the two treatment regimens, and the reason may be due to the limited number of patients included in our study and a possible type II error. We also compared two active treatment regimens in our study, and this may have diminished a possible effect of RAS-blockade. To our knowledge the effect on adipokines between CCB and ARBs has not previously been investigated in a crossover design.

Subclinical chronic low-grade inflammation has been suggested to have a role in insulin resistance and development of type 2 diabetes mellitus, and correlation between pro-inflammatory biomarkers (CRP, IL-6 and TNF-α) and glucose dys-regulation, obesity and atherosclerosis has been shown[256]. No significant difference was seen in these parameters in the GOAAL study, but this may be due to a too small sample size to detect these possible differences during only 8 weeks treatment. Reduced fibrinolytic activity has also been associated with abdominal obesity and insulin resistance[257], but no significant difference in the PAI-1 activity was seen between our two treatment regimens.

We observed a consistent trend towards lower viscosity as vasodilatory treatment was intensified (baseline-amlodipine 5 mg-amlodipine 10 mg- losartan 100 mg + amlodipine 5 mg). Previous studies from our group have shown that WBV is negatively related to insulin sensitivity[231], but no significant difference in WBV and correlation with the improved insulin sensitivity was found between the two treatment regimens in the GOAAL study. The difference in WBV found from baseline and open-label amlodipine 5 mg treatment to treatment with both amlodipine 10 mg and losartan 100 mg + amlodipine 5 mg is probably related to the reduction in blood pressure, but also a significant reduction in haemoglobin level seen from baseline and open-label treatment to the treatment regimen with losartan may explain the reduced viscosity.

The relationship between glucose intolerance and hypokalaemia has been known for decades and was originally proposed by Conn to explain the apparent diabetic state found in primary aldosteronism[103]. The insulin secretory response of pancreatic β-cells to glucose is known to be decreased during hypokalaemia[258], and prevention of hypokalaemia after treatment with ACEIs or ARBs may explain the improvement of insulin sensitivity and the lower risk of diabetes development seen with these drugs. However, no significant difference in measured potassium was seen in the GOAAL study after additional ARB treatment, but this may of course again be related to power of the study and potential type II error.

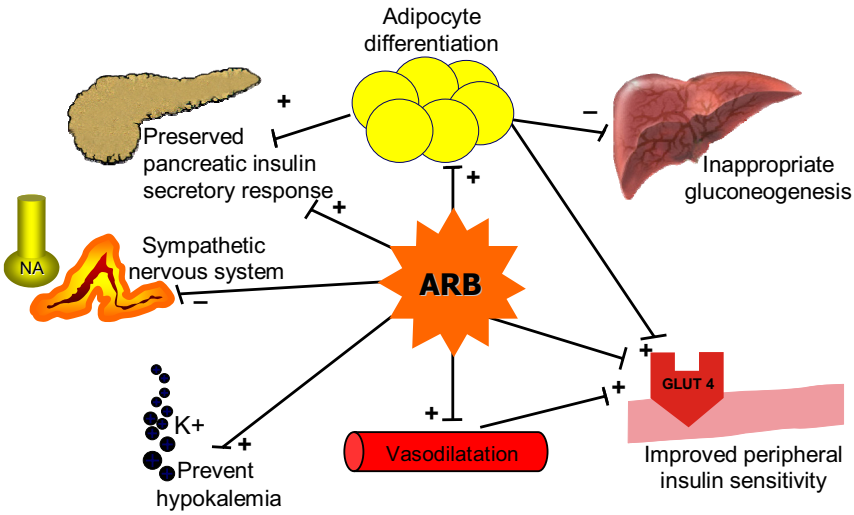
The difference in insulin sensitivity seen in our study may be due to different effects on the sympathetic-parasympathetic balance by our two treatment regimens. Our findings of significant lower venous plasma noradrenaline levels after additional ARB treatment with losartan support this assumption. According to the hypothesis of Julius et al.[68], enhanced sympathetic activity is the primary factor to be associated with hypertension, insulin resistance and possibly obesity. Sympathetic stimulation can cause insulin resistance, and insulin resistance can reciprocate sympathetic stimulation, and a vicious cycle evolves in which the components reinforce each other[68]. Enhanced sympathetic tone can cause peripheral insulin resistance by β-adrenergic stimulation[259], by conversion to more fast-twitch insulin-resistant muscle fibres[260], by decreased capillary density[198] and/or by α-adrenergic vasoconstriction[89, 261-263]. As treatment with the CCB amlodipine has shown to increase sympathetic activity in some previous studies[112, 204, 264-267] and blockers of RAS may reduce sympathetic activation[9], an effect on the SNS may be a plausible explanation for our results. However, no significant differences were seen in HRV, BRS or plasma adrenaline. Other studies have also shown that an increase in noradrenaline concentration with amlodipine is not associated with an increase in adrenaline levels[265], suggesting that there is a clear dissociation

between the activation of sympathetic nerves and of the adrenal medulla. Although, no significant differences in HRV and BRS after treatment with an ARB in our study-population; there was a trend towards higher HF (representing vagal tone), and a lower LF (representing sympathetic with vagal tone), and therefore a lower LF/HF spectral power ratio measured, suggesting a shift in the sympatovagal balance towards less sympathetic drive after treatment with an ARB. It has been suggested that abnormalities in cardiac parasympathetic regulation precede impairment of blood vessel sympathetic control[268], and as we expected differences primarily in the SNS this may be one explanation why we did not find an effect on HRV and BRS in a short time study like this. It is also possible that a potential effect of the ARB treatment was attenuated by the sympathetic activation induced by the open label CCB amlodipine.

There are other possible mechanisms for the improved insulin sensitivity and the reduced new-onset diabetes mellitus seen with blockers of RAS, which are not investigated in our study. Endothelial dysfunction and microvascular changes may affect insulin sensitivity, and endothelial dysfunction is common in patients with essential hypertension[269]. RAS blockade with ACEIs or ARBs has improved endothelial dysfunction and microcirculation in hypertensives[253, 270, 271] and may be one other reason for the reduction seen in diabetes development after treatment with these drugs. Reduction of angiotensin II's potential toxic effects on pancreatic islet morphology and blood supply is another alternative mechanism[272, 273]. It has also been reported that the ARB telmisartan has partial PPAR-γ agonistic effects in concentrations achievable with oral doses recommended for treatment with hypertension[55, 172]. This suggests an insulin-sensitising effect of at least telmisartan like the antidiabetic drugs thiazolidinediones. The ARB losartan used in the GOAAL study has two active metabolites; EXP3174 is the main antihypertensive metabolite and EXP3179 has a more unknown role[274]. It has recently been shown that the metabolite EXP3179 induce partial PPAR-γ activation in vitro, which may explain the beneficial metabolic effects of losartan observed[274]. However, to my knowledge no clinical studies have reported PPAR-γ effects after losartan treatment in recommended antihypertensive doses[172, 274]. Possible mechanisms for this insulin-sensitising ability by ARBs may be through augment in the number of the glucose transporter GLUT-4 and energy metabolism in adipose tissue and peripheral tissue[275, 276]. These intracellular mechanisms including cell culturing have not been investigated in the GOAAL study. A possible insulin-sensitising effect of ACEIs mediated through increased bradykinin levels has also been suggested[277], however this may not be the sole explanation of the beneficial effect of RAS blockers as ARBs (without bradykinin-effects) also have shown effects on insulin

sensitivity and reduction in diabetes development. Recently in a systematic review by Matchar et al.[278], the authors compared the effectiveness of ACEIs and ARBs in treating hypertension and concluded that ACEIs and ARBs, with exception of cough, do not have clinically meaningful differences in benefits or harms including diabetes development. Figure 5 summarises some of the possible mechanisms for reduction in new-onset diabetes mellitus seen with RAS blockers. The anti-diabetic mechanism is probably complex, but based on our results a possible effect on the SNS is our main hypothesis.

Figure 5: Possible mechanisms for preventing diabetes mellitus with ARBs
(*Modified from Aksnes TA et al."The role of ARBs in the management of hypertension" One Way Publishing, 2006:41)*



8.2.3 Diabetes development in hypertension - does it matter?

Quantification of the total health effects of diabetes mellitus and diabetes development is complicated. Diabetic patients often have and die of other diseases, especially cardiovascular diseases, and this may not be registered in e.g. epidemiological studies using only mortality rates. Patients with diabetes mellitus also often have other risk factors, which complicate the picture. The risk of cardiovascular morbidity and mortality also increases continuously with increasing blood glucose concentration and a diagnostic threshold of diabetes may be looked

upon as arbitrary[279, 280]. It has been suggested that treatment-related new-onset diabetes mellitus may not have the same adverse prognostic effect as "spontaneously" occurring diabetes. This view has been based on the apparent "innocent" nature of new-onset diabetes as there has not been seen any strong association between the incidence of new-onset diabetes and an excess risk of hard endpoints in trials[92, 139, 281]. However, cardiovascular complications due to new-onset diabetes may first develop long after the follow-up period, and these randomised clinical trials have not been designed for these purposes[35, 40]. In our results, despite the short time of observation, we found a significantly increased risk of cardiac morbidity in patients with new-onset diabetes mellitus compared to patients without diabetes during the average 4.2-years of follow-up in our pre-specified analyses[282] (Paper V). Patients with diabetes at baseline had as expected the highest cardiac morbidity and mortality rates, but the patients with new-onset diabetes also had increased cardiac morbidity defined as myocardial infarction and heart failure, compared to patients without diabetes mellitus.

There was especially an increase in heart failure in the patients with diabetes compared to the patients without diabetes, both baseline and new-onset diabetes. Possible mechanisms may be more coronary heart disease and diabetic microangiopathy or cardiomyopathy in these patients. Another hypothesis may be that this is related to more atrial fibrillation in this high-risk patient group[282]. Also increased glucose levels below the threshold of the diabetes diagnosis may increase the risk of heart failure. For example in the ONTARGET/TRANSCEND trials a 1 mmol/L higher fasting plasma glucose predicted a 1.10-fold-increased risk of hospitalisation for congestive heart failure (1.09-1.12, *p*-value<0.0001)[283]. It has been hypothesised that prediabetic individuals might have an atherogenic pattern of risk factors even before the onset of clinical diabetes, thereby explaining the relative lack of an association of macrovascular complications with either glycaemic severity or disease duration[43]. However, the concept of "prediabetic" is also controversial in itself as it is only provable in retrospective analyses[284].

There was an increased event rate of stroke in the patients with diabetes at baseline, but no difference in stroke was found between patients with new-onset diabetes and patients without diabetes in the VALUE trial (Paper V). In the 28-year Swedish follow-up study of hypertensives, the risk of stroke was significantly higher in patients with diabetes at entry, whereas this was not the case in patients who developed diabetes mellitus during the first 10 years of follow-up[95]. However, in multivariate regression analyses the development of diabetes mellitus during the whole study was significantly associated with an increased

relative risk of stroke of 67% (1.1-2.6, *p*-value<0.05), indicating it may take time before the increased risk of stroke is showing.

Somewhat unexpected we found decreased over-all and cardiac mortality in the patients with new-onset diabetes compared to the patients without diabetes (Paper V). This is probably due to statistical issues as it is difficult to assess mortality in the patient group with new-onset diabetes mellitus in our study because by definition fatal event in this group could only occur after the time of the diabetes diagnosis. This makes it difficult to compare mortality rate with the other two groups (baseline diabetics and never diabetics), where deaths could be counted throughout the trial. This short duration of follow-up and the limited number of events in the new-onset diabetes population (e.g. only 20 cardiac deaths registered in the new-onset diabetes group) makes the results vulnerable for chance findings. On the other hand when looking at these observational data, more aggressive treatment was reserved by the VALUE investigators to the patients with new-onset diabetes (more aspirin, beta-blockers, diuretics and statins), and this intensive treatment of cardiovascular risk factors may also has reduced mortality.

Long-term observational studies have shown higher incidence of cardiovascular complication in patients having developed diabetes during antihypertensive treatment (predominantly with diuretics and/or beta-blockers)[32, 93, 95, 285, 286]. However, in the 14-year follow up of the SHEP-study newly occurring diabetes among actively treated patients (the diuretic chlorthalidone plus eventually the beta-blocker atenolol) was not associated with increased mortality[98]. These long-term results were retrospective, achieved in elderly patients and the authors used an administrative database to adjudicate vital status and cause of death, which may diminish the impact of the results. The SHEP-study was also placebo-controlled and the results can also be interpreted as a favourable effect of better blood pressure control per se and indicates that any treatment is better than no treatment or control, even when a non-metabolically optimal therapy is used. In a post-hoc analysis from the ALLHAT study a significant increased HR of 1.64 (1.15-2.33) for coronary heart disease was found in patients with incident diabetes, but no significant effects on mortality and other endpoints were found[97]. Potential adverse metabolic effects of diuretics and beta-blockers may partly offset the beneficial effects of blood pressure reduction on overall cardiovascular risk reduction. To ensure maximal cardiovascular protection to hypertensive patients, it seems essential to clarify the cardiovascular risk associated with new-onset diabetes mellitus as done in the VALUE trial.

The VALUE trial has some limitations when evaluating the impact of new-onset diabetes. All the patients are hypertensives with a relatively high cardiovascular risk, and the patients were of mainly Caucasian origin. This limits the possibility to generalise and caution is needed when extrapolating from our results into other ethnic groups. The mortality data must be evaluated with caution as we have included all clinical events during the trial period for the patients in the pre-specified new-onset diabetes group; also events happened before the actual diagnosis of new-onset diabetes. This was done due to knowing that the risk of cardiovascular disease is increased long before the clinical diagnosis of diabetes[35].

8.3 Future perspectives

In view of the predicted increase in the number of diabetic patients during the coming decades[287], it is of great importance from both a medical and an economical perspective to reduce the healthcare burden with focus on prevention, early detection and treatment of both the glycaemia and co-morbidities. Many patients with type 2 diabetes mellitus are unknown of their risk, and their diabetes diagnosis. Blood glucose can rise to diabetic levels with little or no symptoms, and the hyperglycaemia may already at the time of the diabetes diagnosis have made great microvascular and macrovascular damage. Hypertensive patients have a special risk of diabetes development, and must be treated as high-risk patients. In the context of clinical trials the development of diabetes mellitus is often treated as a yes-no variable, defined by which side of the glucose diagnostic threshold each subject ends up. However, the important task is not only to provide that fewer patients are "crossing the line" into diabetes, but to find methods to slowing the rate of the failing pancreatic β-cell and the atherogenetic process in high-risk patients. Lifestyle changes with a combination of appropriate diet, exercise and abstinence from smoking could substantially lower the risk of both hypertension and diabetes development[36, 51, 52, 288]. Good blood pressure control and optimising other cardiovascular risk factors may also reduce the total risk of the patients. Patients with hypertension have a high total cardiovascular risk, and it's of importance to prevent development of diabetes and the accelerated risk of atherosclerosis. Avoidance of medication that could cause weight gain, impair weight loss and/or impair insulin sensitivity or glucose tolerance may be important in high-risk patients. RAS blockers have shown promising results in preventing or at least postponing the diabetes development, and may improve glycaemic control and insulin resistance. However, more research is needed to determine the effect and possible mechanisms.

9. Conclusions

The main conclusions in the present work can be summarised as follows:

- Easily accessible predictors can identify hypertensive patients at risk of developing type 2 diabetes mellitus. Blood glucose and BMI are the most important predictors.
- Antihypertensive treatment with additional AT1-receptor blockade can improve insulin sensitivity compared to treatment with CCBs in patients with hypertension and other cardiovascular risk factors.
- No significant differences were found in adipokines, inflammatory variables and whole blood viscosity after additional treatment of hypertensive patients with AT-1 receptor blockade compared to treatment with CCB alone.
- One possible mechanism for the improved insulin sensitivity by AT1-receptor blockade may be effects on the sympathetic nervous system.
- High risk hypertensive patients who developed diabetes mellitus had increased cardiac morbidity – increased risk of myocardial infarction and heart failure - compared with patients that did not develop diabetes mellitus.

10. References

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