Stiffness and aneurysm of the aorta. Relations to vascular aging, hyperglycemia, and inflammation.

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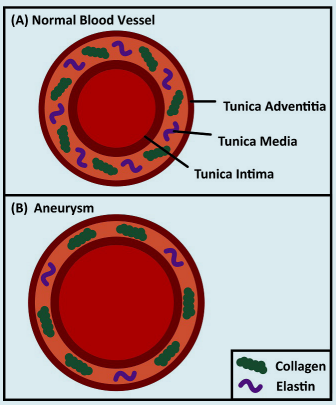

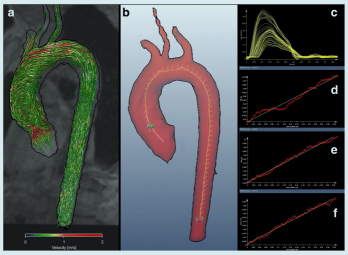

Stiffness and aneurysm of the aorta

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Relations to vascular aging, hyperglycemia, and inflammation

Soumia Taimour

DOCTORAL DISSERTATION

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Title and subtitle Stiffness and aneurysm of the aorta – relations to vascular aging, hyperglycemia, and inflammation

Abstract

A well-functioning arterial system for the transport of oxygen to the cells is essential for human health. The aorta has a key role for the provision of this physiological effect. Disturbances to aortic function can lead to major symptoms and even death due to ruptured abdominal aortic aneurysm (AAA). Multiple diseases may exacerbate, or alleviate the symptoms of aortic conditions.

This thesis examines the following features potentially affecting aortic function:

Aortic stiffness; the wall of the aorta loses its elasticity and blood travels with excessive speed

Horizontal/radial expansion of the abdominal aorta, referred to as AAA

Diabetes mellitus (DM), excessive concentration of glucose in the blood stream

The above relationships are complex and the pathways not yet fully clarified by contemporary medical research. Research has implied a *positive* link between type 2 DM (DM2) and stiffening but a *negative* link between type DM2 and AAA, indicating a need for more studies about details in these correlations. The purpose of this thesis has been to investigate relationships between:

DM2 and aortic stiffness

Biomarkers and AAA

DM2 and AAA

The research has used data obtained from a number of primary sources:

The cardiovascular arm of the Malmö Diet Cancer Study, and the Malmö Offspring Study

National and regional diabetes registries

The Swedish Vascular Register, Swedvasc

The ultrasound screening program for AAA in Malmö

Statistical calculations have been used with the concept of significance as the pivotal measure of whether an association could be proven or not. This has yielded a total of five papers, all published in scientific journals with various co-authors. The main conclusions are:

DM2 is related to aortic stiffness, primarily in older individuals.

None of six evaluated plasma biomarkers predicted aortic diameter 15 – 20 years later

The reported protective effect of DM2 on AAA could not be detected in the early stages of DM2

During follow-up after elective endovascular AAA repair, DM2 was associated with lower need of re-intervention

During follow-up after acute AAA repair, DM2 was associated with lower rates of cardiovascular diseases and mortality

Plausible explanatory theories for the above mechanisms are discussed, and further research is needed to identify which of these that are most clinically relevant.

Key words Hyperglycemia, Aortic Stiffness, Abdominal Aortic Aneurysm

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Cover pictures

Left: Changes in aorta wall protein composition from healthy (top) to dilated (bottom) state. From Mantella LE, Chan W, Bisleri G, Hassan SMA, Liblik K, Benbarkat H, et al. The use of ultrasound to assess aortic biomechanics: Implications for aneurysm and dissection. Echocardiography. 2020 Nov;37(11):1844-50.

Right: Visualisation of magnetic resonance imaging for calculation of pulse wave velocity. From Harloff A, Mirzaee H, Lodemann T, Hagenlocher P, Wehrum T, Stuplich J, et al. Determination of aortic stiffness using 4D flow cardiovascular magnetic resonance - a population-based study. J Cardiovasc Magn Reson. 2018 Dec;20(1):1-11.

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To my parents, Akhtar and my daughters

Table of Contents

Abstract

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This thesis examines the following features potentially affecting aortic function:

- Aortic stiffness; the wall of the aorta loses its elasticity and blood travels with excessive speed
- Horizontal/radial expansion of the abdominal aorta, referred to as AAA
- Diabetes mellitus (DM), excessive concentration of glucose in the blood stream

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Plausible explanatory theories for the above mechanisms are discussed, and further research is needed to identify which of these that are most clinically relevant.

Abbreviations

AAA	Abdominal aortic aneurysm
ADAM	A disintegrin and metalloproteinase
ADMA	Asymmetric dimethylarginine
AGE	Advanced glycation end products
AHT	Anti-hypertensive treatment
ALD	Aldosterone
AMI	Acute myocardial infarction
ANDIS	All new diabetic patients in Skåne
APC-PCI	Activated protein C - protein C inhibitor
AU	Arbitrary units
AVP	Arginine vasopressin
BH2	Bihydrodiopterin
BH4	Tetrahydrodiopterin
BMI	Body mass index
cfPWV	Carotid femoral pulse wave velocity
CI	Confidence interval
CIMT	Carotid intima media thickness
CT	Computer tomography
CPT	Copeptin
CRP	C-reactive protein
CV	Cardiovascular
Cyst C	Cystatin C
DAG	Diacylglycerol
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DPP	Dipeptidyl peptidase-4 Inhibitor
DPP-4	Dipeptyl peptidase-4
E	Elasticity
EL	Endothelin
ECM	Extracellular matrix
eNOS	Endothelial nitric oxide synthase
ET	Endothelin
EVAR	Endovascular aneurysm repair
FPG	Fasting plasma glucose
GLP-1-RA	Glucagon-like peptide 1 Receptor Agonist
HbA_{1c}	Glycated hemoglobin a1c
IFN	Interferon
IGFBP	Insulin-like growth factor-binding protein
IL-6	Interleukin 6
ILT	Intraluminal thrombus
IQR	Interquartile Range

K	Stiffness (change in pressure divided by change in volume)
LISA	Longitudinell integrationsdatabas för sjukförsäkrings- och arbetsmarknadsstudier
Lp	Lipoprotein
Lp-PLA2	Lipoprotein phospholipase 2
MACE	Major adverse cardiovascular events
MCP-1	Monocyte chemotactic protein-1
MDCS	Malmö diet cancer study
MET	Metformin
MDPI	Multidisciplinary Digital Publishing Institute
MMP	Matrix metalloproteinase
MOS	Malmö offspring study
MR-proADM	Mid-regional pro-adrenomedullin
MR-proANP	Mid-regional pro-atrial natriuretic peptide
NDR	National diabetes registry
NPC2	Niemann-Pick disease type C2
PIO	Pioglitazone
PKC	Protein kinease C
PLA	Phospholipase
PNT	Proneurotensin
PP	Pulse pressure
PWV	Pulse wave velocity
RA	Receptor agonist
ROS	Reactive oxygen species
RR	Relative risk
SAF	Skin autoflourescence
SBP	Systolic blood pressure
SCB	Statistics Sweden
SNS	Sympathetic nervous system
SIRT 1	Sirtuin 1
SGLT	Sodium-glucose cotransporter 2 inhibitors
SGLT-2	Sodium-glucose transport protein 2
SU	Sulfonylurea
suPAR	Soluble urokinase plasminogen activator receptor
Swedvasc	Swedish vascular registry
TGF β	Transforming growth factor β
TNF α	Tumour necrosis factor α
US	Ultrasound

List of Publications

Paper I

Taimour S, Gottsäter A, Jujic A, Nilsson PM. Hyperglycemia and arterial stiffness across two generations. *J Hypertens.* 2021; **39**(3):471-5.

Paper II

Taimour S, Zarrouk M, Holst J, Melander O, Engström G, Smith JG, Gottsäter A. No relation between biomarkers at age 47–49 and aortic diameter after 14–19 years of follow-up – a population-based study. *Vasa*. 2017; **46**(4):291-5.

Paper III

Taimour S, Zarrouk M, Holst J, Rosengren AH, Groop L, Nilsson PM, Gottsäter A. Aortic diameter at age 65 in men with newly diagnosed type 2 diabetes. *Scand Cardiovasc J.* 2017; **51**(4):202-6.

Paper IV

Taimour S, Avdic T, Franzén S, Zarrouk M, Acosta S, Nilsson P, Miftaraj M, Eliasson B, Svensson AM, Gottsäter A. Survival, cardiovascular morbidity, and reinterventions after elective endovascular aortic aneurysm repair in patients with and without diabetes: A nationwide propensity-adjusted analysis. *Vasc Med*. 2019; **24**(6):539-46.

Paper V

Taimour S, Franzén S, Zarrouk M, Acosta S, Nilsson P, Miftaraj M, Eliasson B, Svensson AM, Gottsäter A. Nationwide comparison of long-term survival and cardiovascular morbidity after acute aortic aneurysm repair in patients with and without type 2 diabetes. *J Vasc Surg.* 2020; **71**(1):30-38.e3.

Introduction

Arterial Stiffness

Background

The arteries (from the Greek word ἀρτηρία or *arteria*) transport red blood cells carrying oxygen to cells in all parts of the human body. The oxygen is inhaled from the air to the lungs and bound to the transport protein hemoglobin.

A healthy artery has a cylindrical shape within which the blood is surrounded by walls divided into the *intima*, *media* and *adventitia* layers, as indicated in Figure 1.

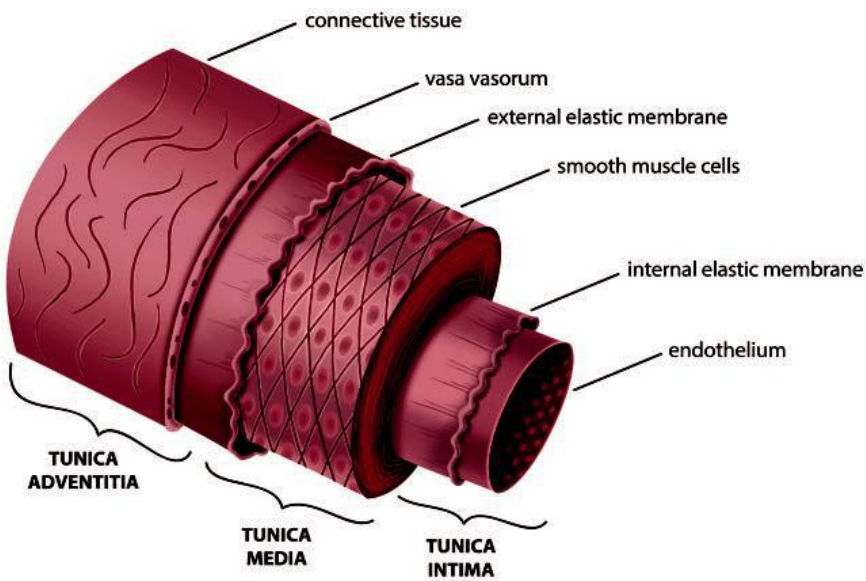

Figure 1. Cross-sectional view of a healthy artery.
Reproduced from [1] with permission from SAGE Publications.

The heart sends pressure waves through the arteries in each contraction. The propagation speed for these pulses depends on the viscoelastic *stiffness* K of the arteries. With P as pressure and V as volume, the definition of K is [2]:

$$K = \frac{\Delta P}{\Delta V}$$

I.e. the pressure change required to cause change in the volume of the vessels. One may observe the inverse relationship to elasticity E.

$$E = \frac{1}{K}$$

This means that the stiffer the walls are, the less will they be able to dilate in response to a pressure change. Stiffness is determined by several factors [3]:

- Transmural distending pressure
- Elastic wall structural components, especially elastin and collagen proteins
- Vascular muscle tone

Pulse wave velocity (PWV), definition

PWV measures the speed at which pressure pulses propagate through the arteries and is an indirect measurement of arterial stiffness [4]. If the walls are stiffer and less elastic, pulse waves travel faster through the vessels as indicated in Figure 2.

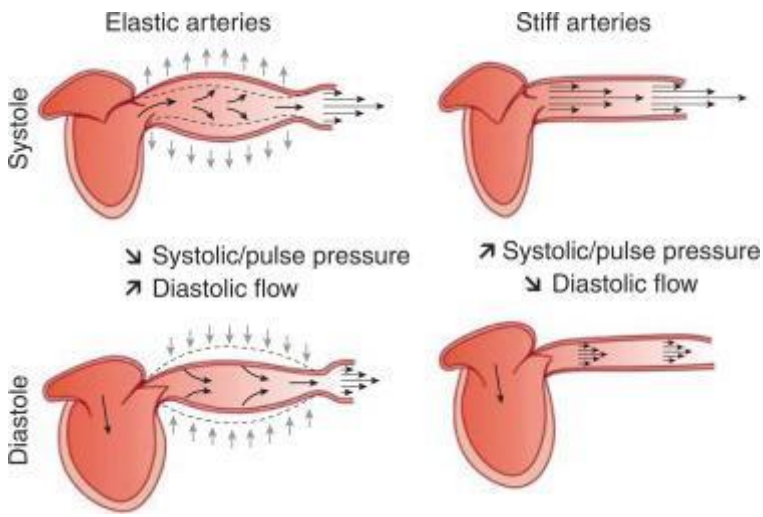

Figure 2. Arterial stiffness and pulse wave velocity.
Reproduced from [5] with permission from Elsevier.

The PWV of a healthy vascular system is highly dependent on age. Based on a definition of a healthy value as PWV < 7.6 m/s, the large Framingham study showed that it becomes increasingly difficult to mitigate vascular ageing when reaching higher seniority [6]. Table 1 summarizes the fraction of the population in an American community defined as arterially stiff.

Table 1. Proportion of population with increased arterial stiffness [6].

Age range (years)	Proportion (%)
50-59	70
60-69	93
70-	99

While there are conflicting results as to whether stiffness differs based on gender, the high dependence on age leads to the formulation of reference values for normal PWV based on age as follows [7]:

- < 50 years: 5-7 m/s
- 50-70 years: 7-9 m/s
- > 70 years: 9-11 m/s

A committee of the European Society of Hypertension has proposed a cutoff value of 9.6 m/s as a threshold for increased cardiovascular risks [8].

Measurement of PWV

Most contemporary commercial instruments estimate the PWV by measuring the time (*Δt)* elapsed between passage of a pulse wave at carotid and femoral artery, respectively, to estimate *Δt* from the right carotid to the right femoral artery (cfPWV). The timer must be triggered at exactly the same phase in the pulse, normally at the "foot" (bottom of the cycle) or at the point of maximum positive slope [9]. The distance (*d*) between the measurement points is then divided by the time difference Δt to obtain the value of PWV defined as

$$PWV = \frac{d}{\Delta t}$$

as displayed in Figure 3.

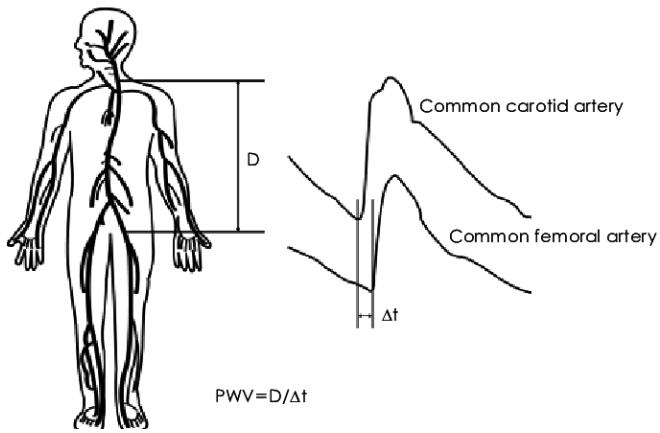

Figure 3. Measurement of carotid-femoral pulse wave velocity.
Reproduced from [10] with permission from Korean Circulation Journal.

As this leads to an overestimation of the real PWV [11], the correct time measure should instead be the so called *intersecting tangent time* (Δt_{it}):

$$\Delta t_{it} = \frac{\Delta t - 14.96}{0.8486}$$

The distance measurement has also been modified, and the method used in the Sphygmocor® device employs a compensation factor of 0.8 [12-13]:

$$PWV = 0.8 * d / \Delta t_{it}$$

Pathology

The apparently linear rise of PWV with age has been referred to as *vascular aging*, most clearly recorded as changes in arterial wall characteristics [14]. The physiological processes manifest behind this very strong age dependence are summarized in Figure 4.

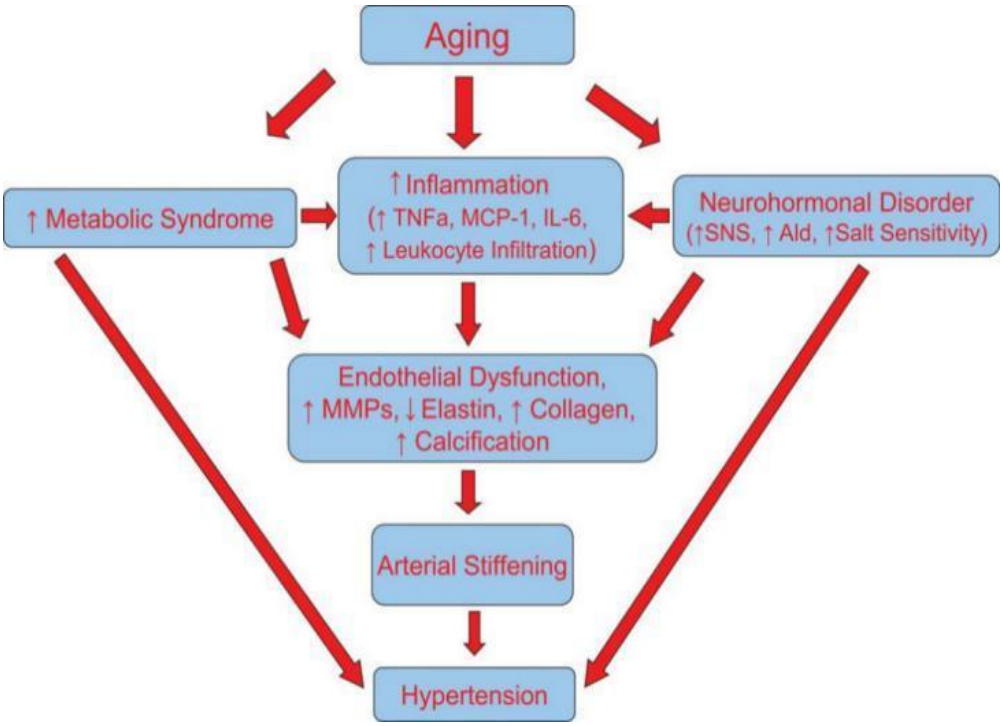

Figure 4. Pathways of age-arterial stiffening. SNS: sympathetic nervous system; Ald: aldosterone; MMP: matrix metalloproteinase; MCP-1: Monocyte chemotactic protein-1; TNFα, tumor necrosis factor alpha; IL-6, interleukin-6.

Reproduced from [15] with permission from Wolters Kluwer Health, Inc.

The stiffness of the arterial walls is in general higher in men than in women and also affected by several lifestyle and health-related factors [16-17], for example:

- High body mass index (BMI)
- High blood pressure
- Smoking
- Type 2 diabetes mellitus (DM2)
- Certain pharmaceutics and therapies, e.g. calcium supplements and calcium-based binders

Factors affecting arterial stiffness are summarized in Figure 5.

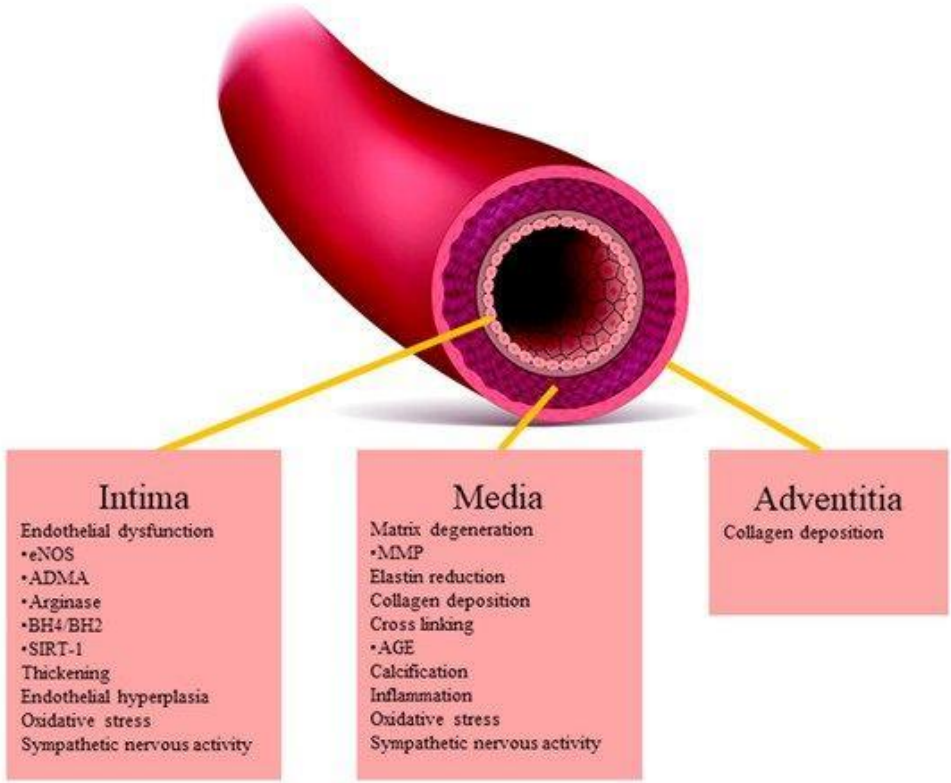

Figure 5. Pathological pathways influencing vascular ageing. eNOS: endothelial nitric oxide synthesis, ADMA: asymmetric dimethylarginine, SIRT-1: Sirtuin-1, BH4: tetrahydrobiopterin, BH2: bihydrobiopterin, MMP: matrix-metalloproteinases, AGE: advanced glycation end product.

Reproduced from [18] with permission from the Multidisciplinary Digital Publishing Institute (MDPI).

Consequences

Stiffening of vessels has been linked to a variety of hazardous and potentially life-threatening conditions such as coronary heart disease [19] and stroke [20]. The link between these conditions is not clear, but two tracks of plausible factors have been identified:

- Stiffening leads to increased pulse pressure (PP). As displayed in Figure 6, the increased pulse speed leads to waves being reflected quicker and through positive interference altering the wave pattern.

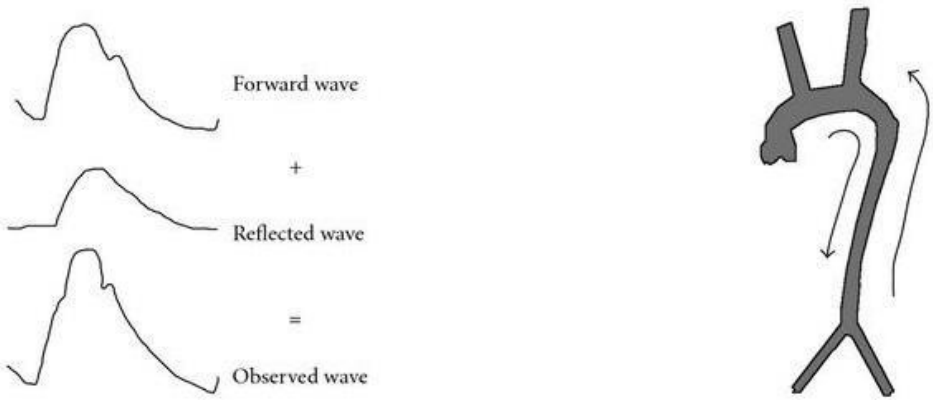

Figure 6. Wave reflections and blood flow.
Reproduced from [21] with permission from Hindawi.

- The amplification of outgoing and reflecting waves leads to a higher systolic blood pressure (SBP) and a lower diastolic blood pressure (DBP), thus an increase in PP [22]. PP has been reported to be positively associated with a number of cardiovascular outcomes such as stroke, acute myocardial infarction (AMI), mortality, and congestive heart failure [23].

Arterial stiffening can also lead to atherosclerosis through several pathways [24]:

- Structural changes to the arterial wall
- Altered currents from reflections leading to shear stress
- Endothelial dysfunction

Treatment

Pharmacological options for the alleviation of arterial stiffness are limited [25]. Antihypertensive treatment (AHT) has been shown to work in some cases but could also lead to adverse effects [26]. In particular inhibitors of the renin-angiotensin system and certain statins have been proposed to be effective [27-28].

In terms of lifestyle changes, frequent physical exercise appears to be the best remedy [29]. Reduction of smoking and sodium intake is also recommended, as reviews of multiple studies have identified these as significant risk factors for arterial stiffness [30-31].

Aortic dilatation and abdominal aortic aneurysm (AAA)

Background

The aorta is the largest artery in a human, transporting oxygenated blood from the heart, with a shape as depicted in Figure 7.

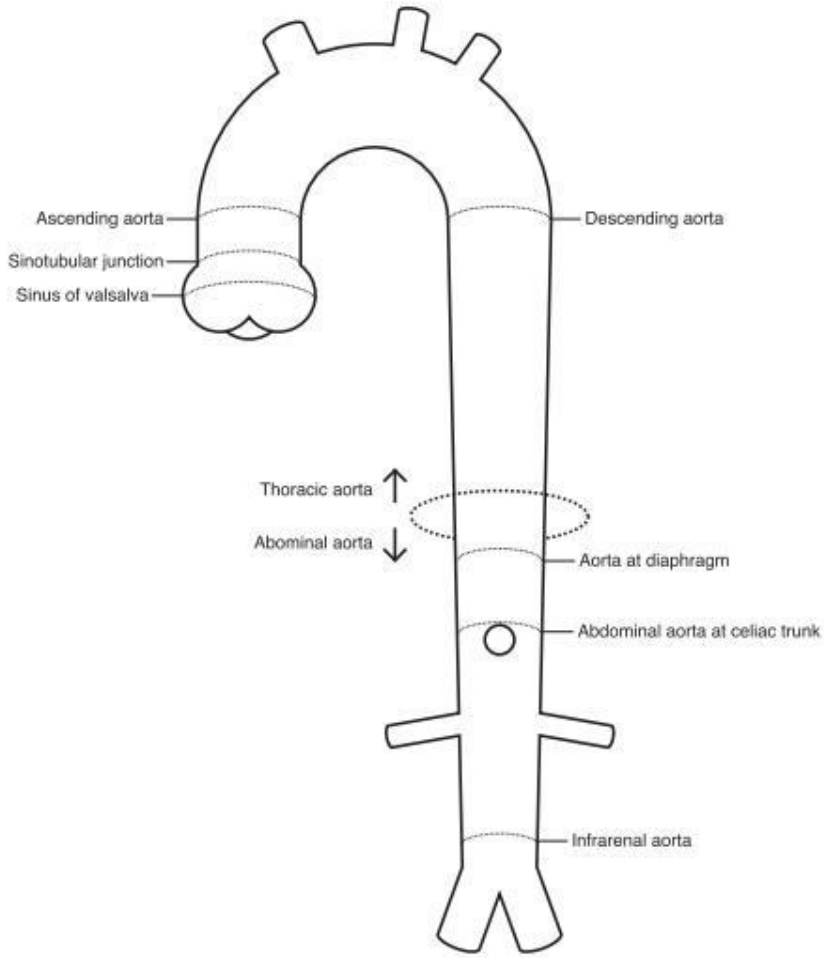

Figure 7. The aorta.
Reproduced from [32] with permission from Oxford University Press.

The mean diameter of the infrarenal aorta in healthy individuals is 19-22 mm in men [33] and 10-15 mm in women [34]. Aortic diameters normally increase with age,

body size, blood pressure, and physical activity [32]. The aortic wall might expand further in the radial directions (dilate) at a localized point in the chest or abdomen.

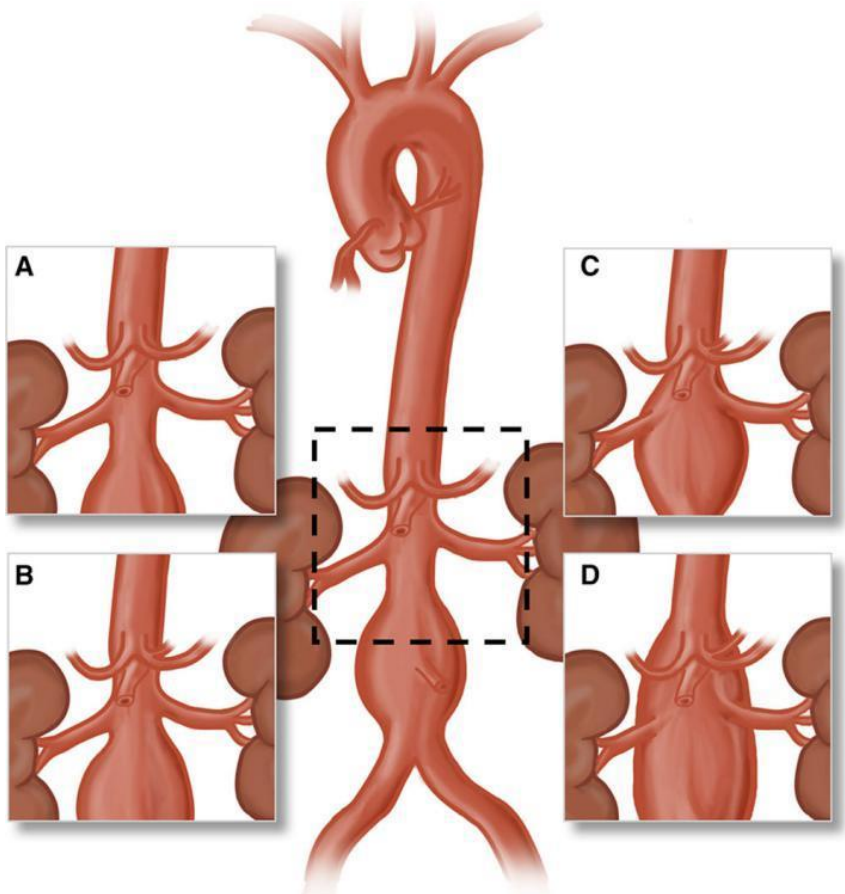

Figure 8. Aneurysm types: A) Infrarenal, B) Juxtarenal, C) Suprarenal, D) Thoracoabdominal.
Reproduced from [35] with permissions from Wolters Kluwer Health, Inc.

As Figure 8 indicates, a dilatation is classified according to where it occurs in relation to the kidneys:

a) Infrarenal – below
b) Juxtarenal – next to
c) Suprarenal – above
d) Thoraco-abdominal – at diaphragm

The enlargement can be an ongoing process ranging for years and even decades. A dilatation of ≥ 30 mm of the abdominal aorta is classified as an abdominal aortic *aneurysm* (AAA) [36]. An aneurysm expanding on both sides is called *fusiform*, whereas it is referred to as *saccular* when only located on one side of the vessel (Figure 9).

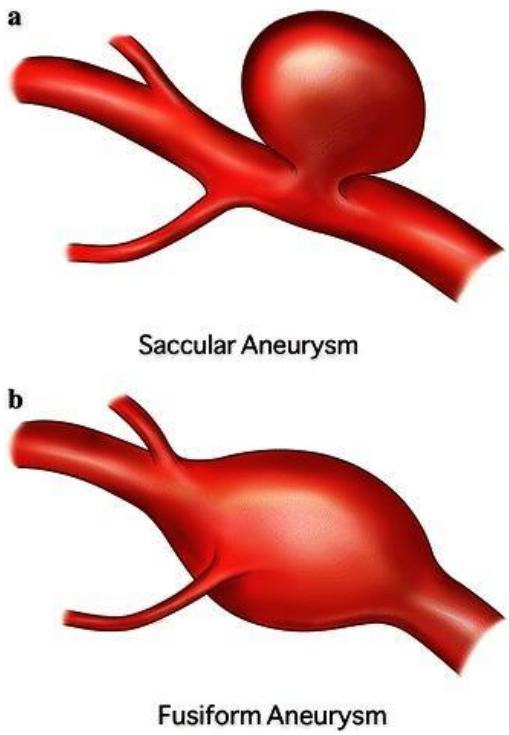

Figure 9. Fusiform (A) and Saccular (B) aneurysm.
Reproduced from [37] with permission from Springer Nature.

Pathology

In the buildup to dilatation, vessel proteins and muscle tissue are often worn down and replaced by basophilic ground substance, mucoids [38]. These processes are often adjoined by inflammation of the aortic walls. Such interplay between a variety of processes is illustrated in Figure 10.

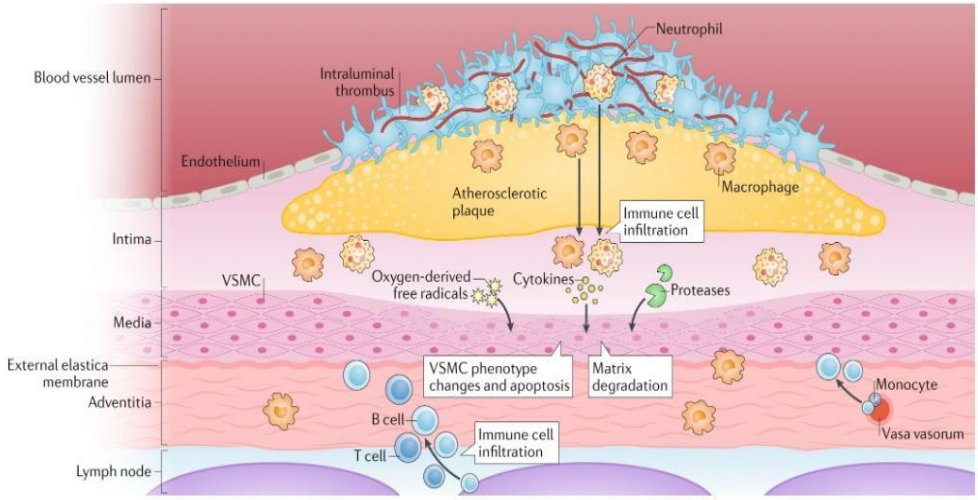

Figure 10. Features contributing to aortic dilatation.
Reproduced from [39] with permission from Springer Nature. VSMC=Vascular Smooth Muscle Cell

Risk markers for AAA include [40- 41]:

- Age
- Male sex
- Smoking
- Hyperlipidemia, atherosclerosis
- Hypertension

Prevalence

Prevalence of AAA has been estimated in several studies over the years, some of which are listed in Table 2.

Table 2. Prevalence of AAA by sex [42-44].

Study location	Age range (years)	Prevalence in men (%)	Prevalence in women (%)	Prevalence overall (%)
Norway [42]	All	8.9	2.2	5.3
Italy [43]	50-75	7.1	4.3	6.1
UK [44]	65-80	7.6	1.3	3.9

The higher rates observed among men has prompted the introduction of ultrasound screening for AAA in all men at 65 years of age in some countries including Sweden [45 - 48].

Diagnostic methods

An AAA is most commonly visualized by measurement of the aortic diameter through ultrasound (US) or computerized tomography (CT) [36]. When screening for AAA in Sweden, the maximal antero-posterior diameter of the abdominal aorta is determined by US, measuring leading edge to leading edge (Figure 11).

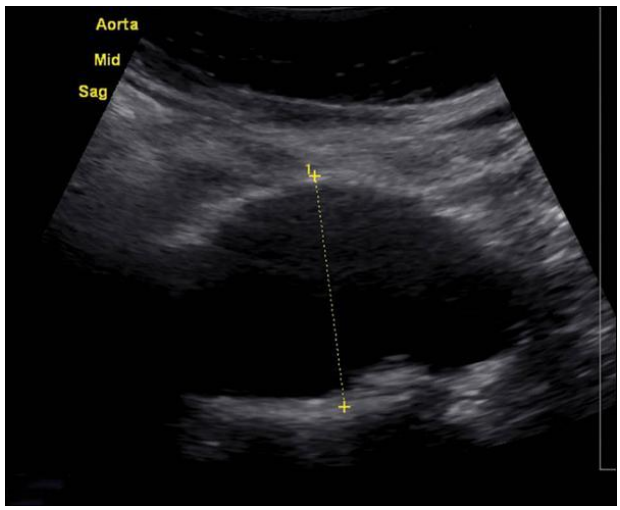

Figure 11. Ultrasound estimation of aortic diameter.
Reproduced from [49*]* with permissions from the American Family Physician Journal.

Consequences

Whereas most moderately enlarged aortas remain unnoticed, some AAA can cause symptoms including pain in the abdomen, lower back, and loins, and pulsations in the abdomen. These symptoms may be life threatening as they sometimes are forthcoming to rupture. Research has shown that surveillance of asymptomatic small aneurysms (4.0 – 5.4 cm) is safe and that early surgery, even when operative mortality is kept low, does not save lives [50-51]. It is well established that the risk of rupture is increased with maximal aneurysm diameter size. An abdominal aortic diameter ≥ 5 cm in women or ≥ 5.5 cm in men are considered the thresholds for

recommending elective operative repair [36], as benefit of elective operative repair is considered to outweigh the risk of non-operative management and rupture.

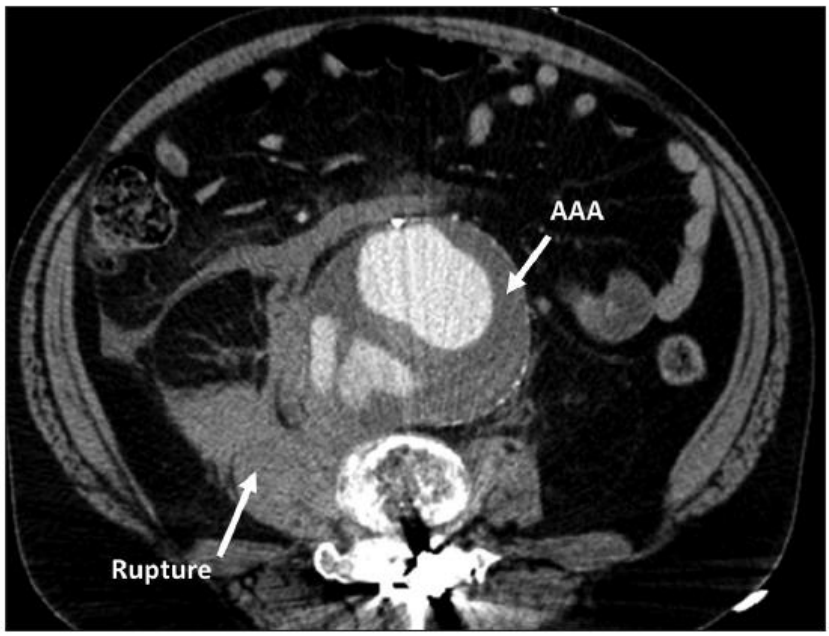

Figure 12. Aortic rupture.
Reproduced from [52] with permission from Radcliffe Vascular.

A ruptured AAA (Figure 12) is a life-threatening condition requiring immediate surgery [36]. Even if the patient survives long enough to be transported to a hospital and undergo surgery, the 30-day rate of fatality is above 30 % [53].

Treatment

Several pharmaceutical remedies have been tested, but a thorough review in 2015 concluded that there is no available medicine to mitigate AAA expansion [54]. In terms of lifestyle changes, the strongest effect for reducing risk of further growth has been reported to come from smoking cessation and blood pressure control [36]. Elective repair for asymptomatic and acute repair for symptomatic AAA, mostly due to rupture, can be performed with two methods: endovascular aneurysm repair (EVAR) or open repair [36].

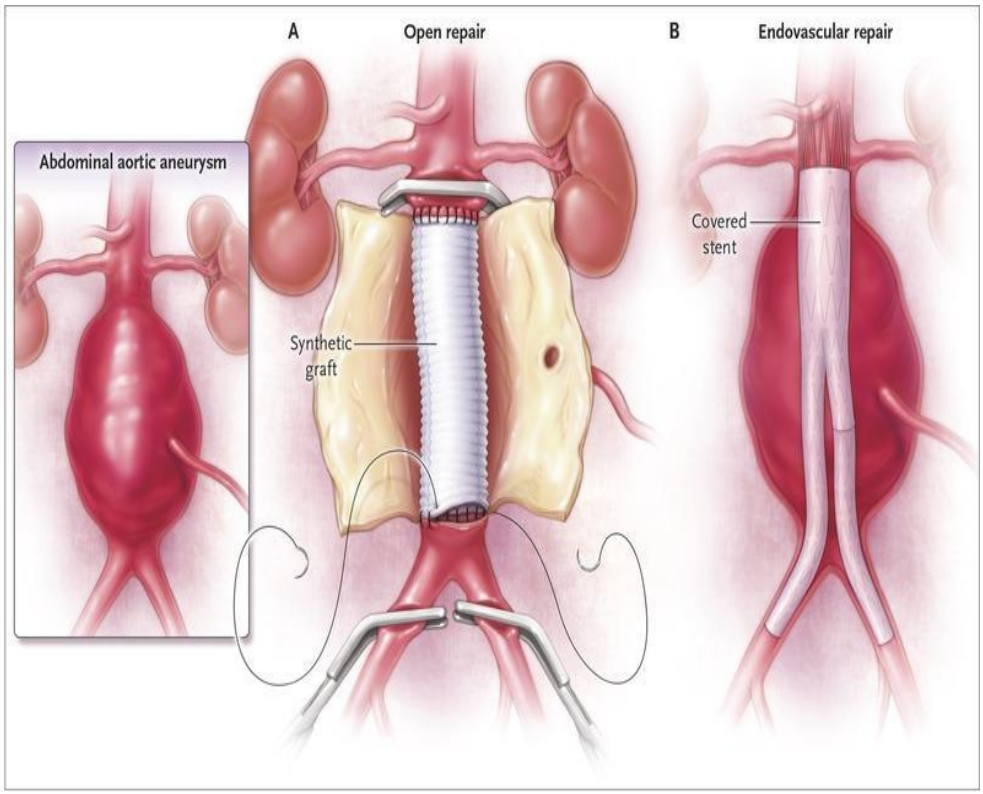

Figure 13. Open vs. Endovascular repair of AAA.
Reproduced from [55] with permission from Massachusetts Medical Society.

EVAR is a process whereby a *stent device*, is inserted through the femoral artery to the dilated section. This device, usually a tube of metal and synthetic material [56], is inserted and inflated in the blood vessel as shown to the right in Figure 13. EVAR carries a lower risk of perioperative mortality (0.5-2 %) than open repair, but is associated with a higher long-term risk of re-interventions [36].

In open aortic repair, the aorta is opened, and an interposition graft is inserted above the bulged section. The aneurysmal dilated section is removed, and the remainder of the vessel is then joined by the graft with suture as shown in the middle part of Figure 13. Open repair is a more invasive technique than EVAR and therefore carries a higher risk of perioperative mortality. The advantage is that the entire aneurysm has been permanently removed, virtually eliminating the risk for future ruptures for all except for rare cases of pseudoaneurysm formation in the anastomosis [36].

Hyperglycemia and type II diabetes

Background

Insulin is an amino acid hormone formed by two peptide chains joined by a disulfide bond [57]. The pancreatic beta cells produce insulin which is secreted into the blood vessels to regulate the absorption of glucose from nutrients to cells in various human organs [58]. Insulin also promotes the conversion to fat and storage of energy reserves, and can diffuse through cell membranes and transport energy as shown in Figure 14.

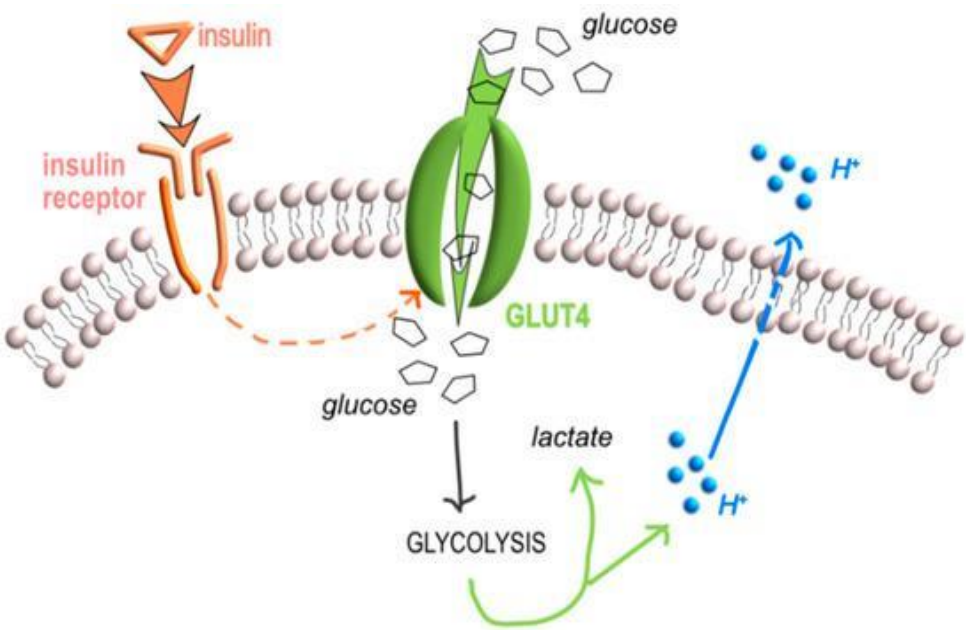

Figure 14. Insulin-mediated transport of energy.
Reproduced from [59] with permissions from MDPI.

A person whose pancreas completely lacks the ability to generate insulin is in a state of type I diabetes (DM1). DM2 patients retain some production of insulin, but in less than adequate amounts. Another common feature is that their cells in general, but most pronounced in skeletal muscle, are incapable of executing the chemical reactions required for the processing of insulin and glucose, i.e. are *insulin resistant* [60].

Prevalence

It is estimated that 9.3 % of the population worldwide suffers from DM1 or DM2, with numbers expected to exceed 10 % by 2030 [61]. Especially DM2 is more prevalent among older people [62]. Diabetes patients in Sweden have been estimated to still have a 15 % higher overall mortality rate than healthy subjects [63] and diabetes is considered to be involved in 5 % of all deaths worldwide [64]. However, it has been recorded that mortality can be reduced significantly with proper follow-up and treatment routines [65].

Measurements

Fasting Plasma Glucose: Glucose is distributed in the extracellular matrix (ECM) and travel with the circulating blood to many organs including muscle cells, nerve cells, and hepatocytes. Fasting plasma glucose (FPG) measures the blood glucose concentration in plasma in mmol/L following a period of at least 8 hours without intake of either liquid or food.

Glycosylated Hemoglobin (HbA1c): Excess glucose in the blood stream can form a chemical bond with oxygen carrying hemoglobin molecules. One of its beta chains, primarily N terminal valine, can be glycated and converted to a stable ketoamin, HbA_{1c} reflecting the share of glycated hemoglobin molecules among the erythrocytes in mmol/mol [66]. It forms an indirect measure of the average glucose concentration over the past few months. The method can be influenced by the longevity of the erythrocytes. If their life expectancy has been reduced from e.g. hemolysis, the method will show a lower value although glucose levels are the same.

Skin autoflourescence (SAF): SAF of advanced glycation end-products (AGEs) refers to the emission of light from the skin specifically emitted by the de-excitation of AGEs. As individuals with diabetes exhibit higher fluorescence than others [67], SAF can be used as a biomarker of diabetes or an indicator of the severity of the diabetic state [68]. SAF is measured in arbitrary units (AU). The average SAF in subjects with diabetes have been demonstrated to be significantly higher than in nondiabetic subjects [69].

The definition of a diabetic state is based on a number of variables related to blood glucose concentration, as defined in Table 3.

Table 3. DM Diagnosis Criteria [70]

Variable	Threshold Value
FPG	7 mmol / l (2-3 separate measurements)
HbA_{1c}	48 mmol / mol
Non-fasting plasma glucose	11.1 mmol / l
PG during Oral Glucose Tolerance Test	11.1 mmol / l

Pathology

In a patient with DM2, the number of insulin receptors on the cell surface has been strongly reduced or are dysfunctional. Instead of being processed in the cells and converted to energy, glucose will stay in the blood stream, causing a higher blood sugar concentration. This state is referred to as *hyperglycemia.* The primary cause of the rapidly increasing rates of DM2 especially in younger people is lack of exercise and consumption of unhealthy nutrition [70, 71]. In older patients, the main factors are genetics, obesity, and inactive lifestyle [72].

Cellular consequences

Several changes at the cellular level have been observed in diabetes. The most important are the following [73]:

- Formation of advanced glycation end products (AGE)

Glucose molecules form chemical reactions with proteins (most notably collagen) and lipids in the walls of the artery in three steps. They bind to a reactive amino chain and form the intermediary Schiff base subsequently converted into an Amadori product. Over time, these are cross-linked and joined to form the end product AGE as shown in Figure 15.

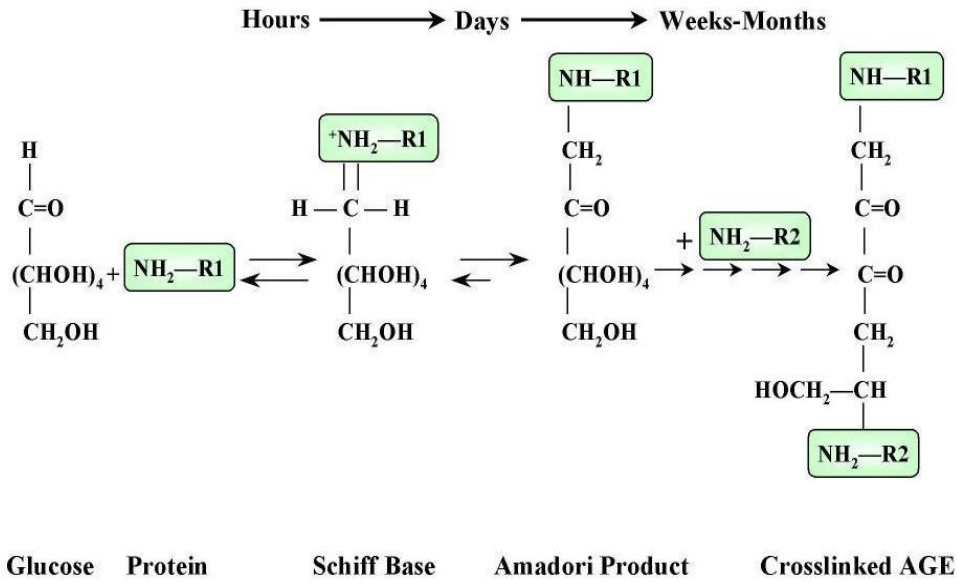

Figure 15. Formation of AGEs.
Reproduced from [73] with permission from Springer Nature.

The most prevalent of these are the carboxymethyl lysine protein adducts [74]. AGEs may bind to cell membrane receptors and cause modifications to cell signals, activation and inhibition of genes and inflammatory substances [75].

- Oxidative stress

DM patients have increased levels of reactive oxygen species (ROS) free radicals [76]. This process takes place through several pathways, including activation of electron transport in the mitochondria and enhanced production of nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase.

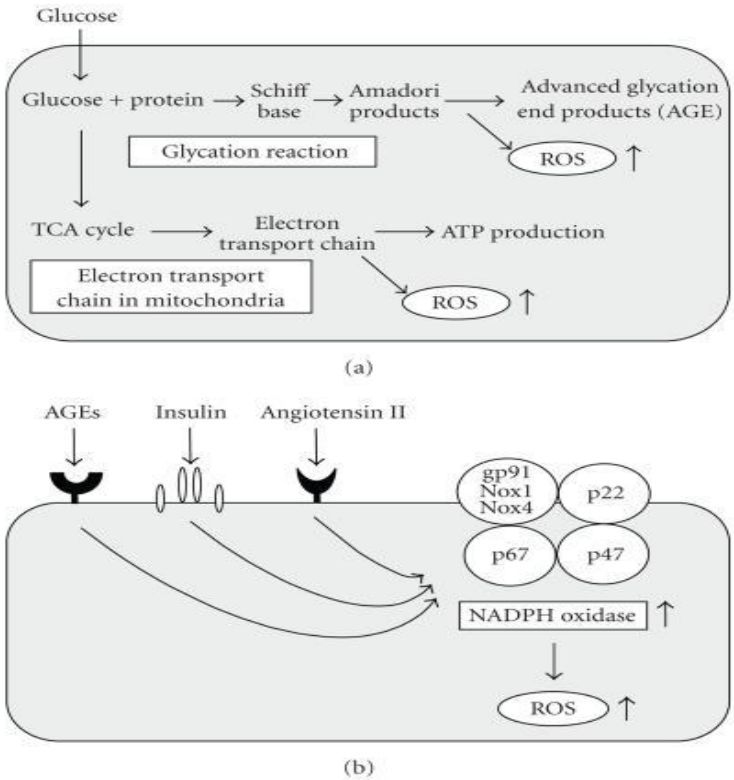

Figure 16. DM2 induced elevation of ROS through a) Direct glucose mediated electron transport and chemical reactions, and b) End product reaction with NAPDH oxidase.
Reproduced from [77] with permissions from Hindawi.

The increasing amounts of ROS radicals may cause falls in nitric oxide levels, inflammation and lipoprotein modifications significantly adding to the risk of atherogenesis [78].

- Protein kinase C (PKC)

The harmful PKC protein has been demonstrated to be activated by excessive amounts of glucose [79]. As Figure 17 illustrates, this takes place through a series of reactions forming the reactant diacylglycerol (DAG). This may in turn bind to a site on the PKC molecule and activate them.

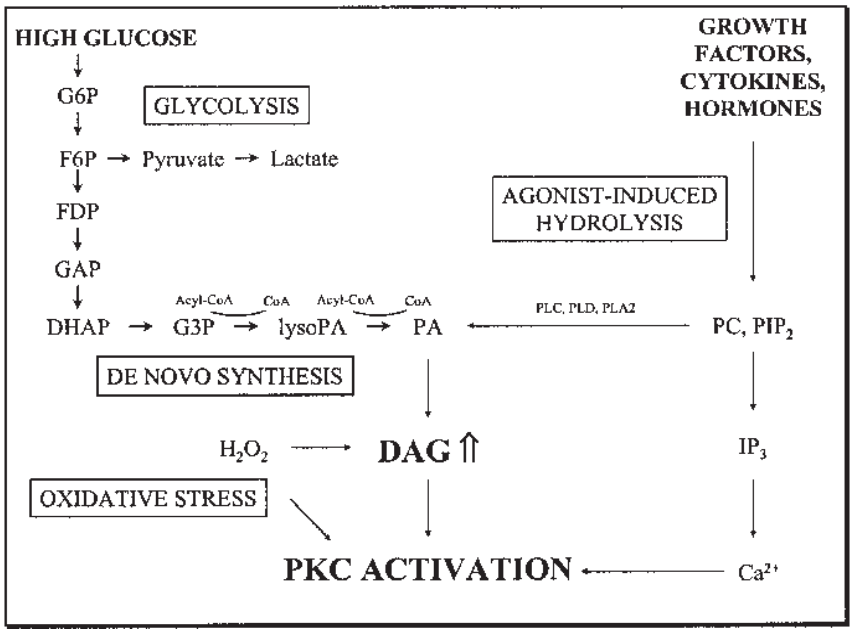

Figure 17. PKC through DAG synthesis.
Reproduced from [80] with permission from SAGE Publishing.

PKC has been associated with increased vascular permeability, endothelial dysfunction and flawed growth signaling [81]. These and other effects may cause several symptoms such as:

- Fatigue
- Polyphagia, polydipsia
- Polyuria and ketonuria
- Impaired vision
- Headache, dizziness
- Weight loss
- Infections
- Poor healing of skin rashes and sores
- Kidney malfunction

In advanced stages, a deteriorating diabetes condition left untreated may accelerate cell deficiencies with consequences including:

- Cardiovascular diseases, e.g. myocardial ischemia
- Stroke
- Retinopathy and blindness
- Terminal kidney insufficiency
- Foot ulcers, amputations

Treatment

Swedish official recommendations state lifestyle changes as the top preventive priority, including dietary changes, more physical activity, and reduction of alcohol consumption as well as cessation of smoking [82]. In general, remedies for DM can be divided in a few categories:

- Decreasing obesity

A high BMI is one of the strongest risk factors for the development of DM2. Based on a thorough review, the American Diabetes Association recommends the reduction of obesity as a target in many treatment schemes. This can be achieved through various means, e.g. eating habits, exercise, medication or surgery [83].

- Pharmaceutics

There are several glucose-lowering drug agents available. The most prevalent in Swedish care is metformin, and others include meglithinides, acarboses, glitazones, sulfonylurea (SU), inhibitors of dipeptidyl peptidase-4 (DPP-4) and sodium-glucose linked transporter 2 (SGLT-2), glucagon-like peptide-1 receptor agonists (GLP-1-RA), and insulin [84]. A proposed treatment algorithm is portrayed in Figure 18.

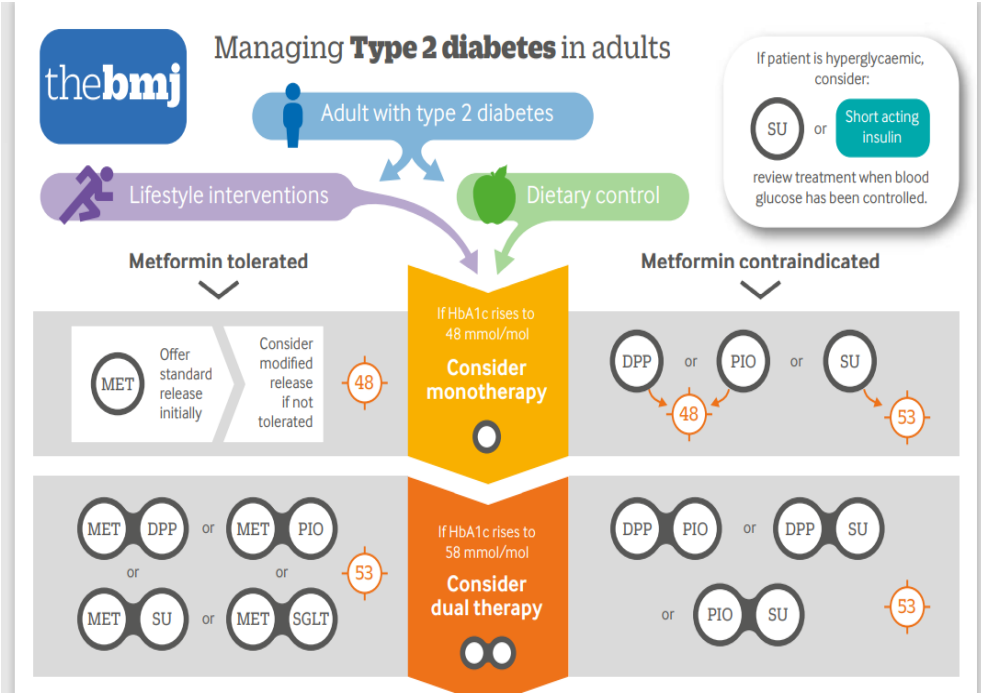

Figure 18. Treatment algorithm for medication approach against type 2 diabetes.
DPP= Dipeptidyl peptidase-4 Inhibitor, MET = Metformin, PIO=Pioglitazone, SGLT = Sodium-glucose cotransporter 2 Inhibitor, SU=Sulfonylurea. Reproduced from [85] with permission from BMJ Publishing Group Ltd.

- Surgery

Surgical procedures might potentially alleviate the dysfunctions present among DM patients. These *metabolic/bariatric* procedures include adjustable band, sleeve gastrectomy and gastric bypass, all aimed at altering metabolism through long-time weight reduction [86]. While surgery is rarely included in official treatment guidelines, a meta- analysis of various surveys showed that bariatric surgery reduced type 2 diabetes significantly [87].

Biomarkers and AAA

Some physiological processes are inherently difficult to monitor, especially in the early stages or during screening for future risks. However, they can often be shown to correlate with phenomena requiring less invasive sampling. Biomarkers are used to detect or indirectly measure the status of a malady by obtaining values of a compound believed to be related to the disease. As mentioned, diabetes can be identified through either of FPG, HbA1c, or SAF.

Most diseases will incur changes in various cell proteins, making proteomics a prime target for biomarker identification [88]. As an example, there is a large number of potential biomarkers showing correlation with heart failure [89-90], and one could use cardiac troponin (cTn), cardiac myosin binding protein (cm) or C-terminal-provasopressin (copeptin) as indicators of AMI [91-93].

For the purpose of this thesis, it was relevant to identify and evaluate proteins that can predict present or future aortic dilatation or aneurysm. Figure 19 identifies the most prevalent processes in AAA formation and the proteomics involved.

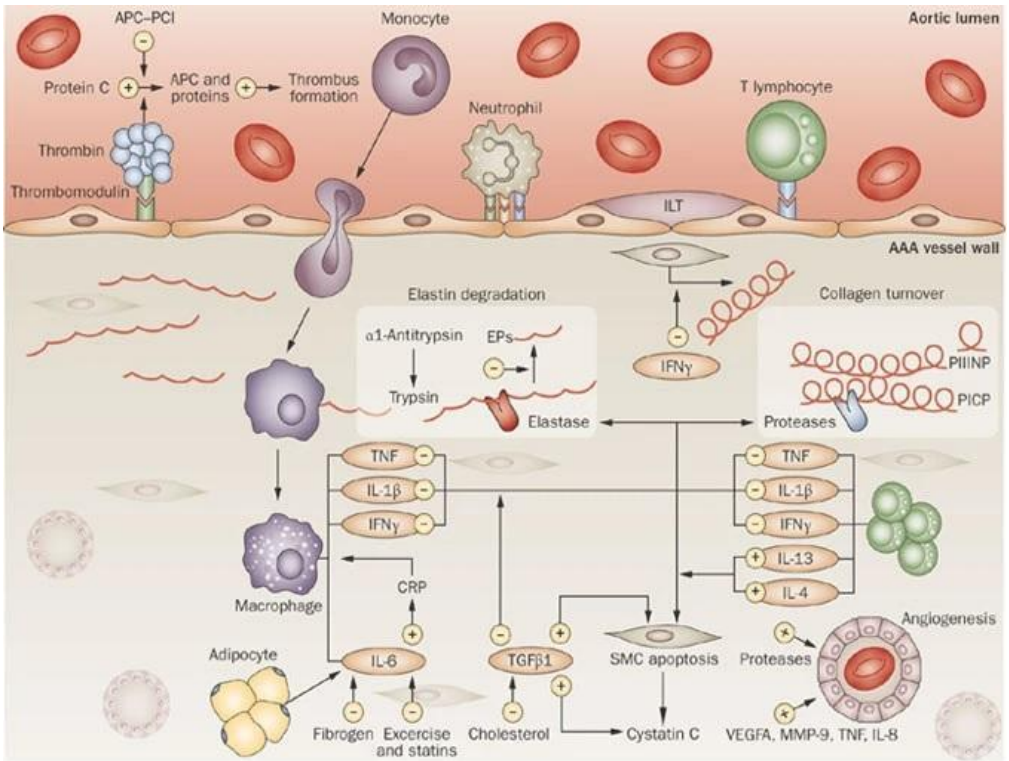

Figure 19. Proteins and potential biomarkers related to AAA development.
Reprinted from [94] with permission from Springer Nature.

A number of these substances have been found to correlate with aortic diameter [95-99], although sex differences in the response of several of the above mentioned proteins make the mapping even more complicated [100]. In this thesis, the following proteins were evaluated as potential markers for AAA:

- C-reactive protein (CRP): Occurring in the blood plasma, binding to dead or dying cells as a part of the immune system [101]. Previous studies have reported a potential or significant link to AAA [102].
- Proneurotensin (PNT): A precursor to neurotensin, a hormone in the central nervous system linked to obesity regulation [103].
- Copeptin (CPT): Has unknown functionality, but exists in the blood plasma with a high correlation to Arginine Vasopressin (AVP) and is easier to measure [104].
- Lipoprotein-associated phospholipase 2 (Lp-PLA2): An enzyme known to be associated with inflammation, incurring cardiovascular risk and diabetic retinopathy [105].
- Cystatin C: A protein present in all body fluid cells and responsible for inhibiting proteinase conditions [106]. Linked negatively to AAA growth [107].
- Midregional proatrial natriuretic peptide (MR-proANP): A natriuretic peptide related to inflammation and hemodynamic stress [108].
- Midregional proadrenomedullin (MR-proADM): A peptide present in lungs, heart, and gastrointestinal regions [108].

Hyperglycemia and aortic stiffness

It has been repeatedly demonstrated that DM patients have significantly higher arterial stiffness values than individuals without diabetes, when measured as PWV [109]. In regression analyses, stiffness markers have been shown to be associated with indicators of hyperglycemia such as HbA_{1c} [110] and FPG [111]. Survival analyses displayed that people with higher arterial stiffness developed diabetes much more rapidly than those with lower stiffness values, indicating reversed causality from stiffening to diabetes [112]. A survey found no less than 28 studies in DM2 and 11 in DM1 confirming this link [113]. The pathology of this relationship is not yet fully determined, but there are several mechanisms [114]:

- Insulin resistance, where excessive concentrations of insulin could increase restructuring of the walls
- Endothelial dysfunction, causing excess oxygen concentrations in vessel walls and unwanted reactions [115]
- AGE, being formed in the walls and cross-linking collagen molecules
- Low-grade inflammation of the wall structure and increased levels of CRP
- Oxidative stress
- Fragmentation of proteins in the walls

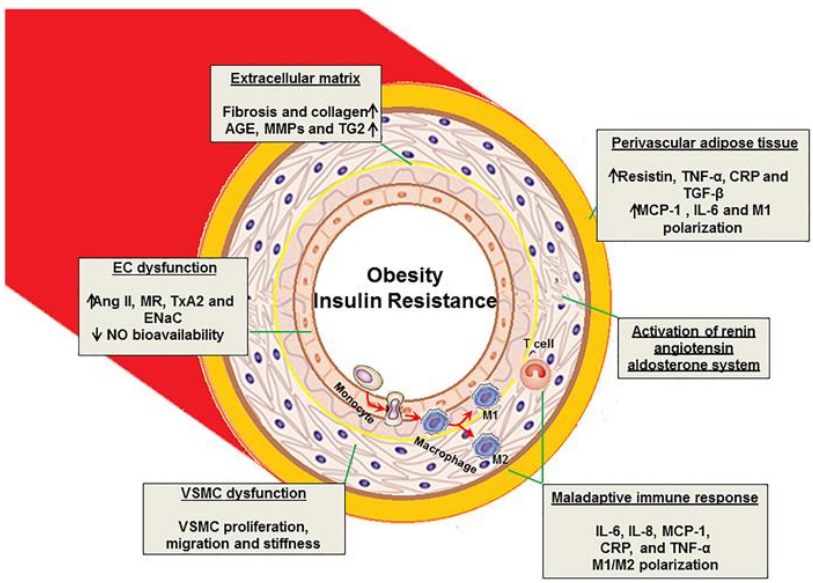

Figure 20. Pathways for arterial stiffening in subjects with diabetes.
Reproduced from [116] with permission from Frontiers in Physiology.

Hyperglycemia and AAA

While the prevalence of diabetes as mentioned is rising steeply [61], rates of AAA have been declining [117]. Whereas this could be attributed to a fall in the rate of smoking, this opposing trend led to the hypothesis that DM patients have a better chance of avoiding AAA and its complications [118, 119], explained by:

- Extracellular matrix (ECM) remodelling: The ECM is an intricate network primarily consisting primarily of membrane, elastin, collagen, and proteoglycans [119]. It has been shown that diabetes leads to a rearrangement of these, altering the balance between their proportions. This yields a 20 % decrease in aortic wall stress, 20 % thicker carotid Intima media thickness (cIMT) and an enrichment of a number of proteins related to cell adhesion [120, 121]. All these changes have been related to a reduced growth of AAA.
- Increased formation of AGE: As mentioned above, the increased concentrations of glucose in the vessels incur glycated oxidation of protein. These processes may produce several end products, including carboxymethyllysine (CML) and carboxyethyllysine (CEL), and pentosidine. It has been identified that patients with large aneurysms have lower values of these AGEs [122], suggesting that they have a protective function against aneurysm growth.
- More inflammation: DM2 is known to promote vascular inflammation through a multitude of processes [123]. Although the mechanisms are not clear, however, it has been argued that increased inflammatory activity could inhibit aortic dilatation [119].
- Reduced neoangiogenesis: The link between DM and angiogenesis is complex and takes place through a variety of mechanisms in opposing directions, where a patient may even experience increased and decreased vessel cell growth in different parts of the body [124]. When looking at the regions around the heart and abdomen, a thorough survey of all links identified inhibition of angiogenesis as the dominant feature [125]. As enhanced angiogenesis has been showed to be a major factor in the development of AAA [126], impaired angiogenesis in the cardiovascular area might lead to slower aortic dilatation.
- Vascular smooth muscle cell (VSMC) homeostasis: DM2 patients are less prone to VSMC apoptosis [127]. But as the death of VSMC cells in fact is a major contributor to aneurysm growth [128, 129], this might again indirectly mean less problems with AAA.

- Enhanced intraluminal thrombus (ILT) formation: Whereas DM is not normally associated with major modifications of the ILT, it has been reported that especially DM2 can lead to changed fibrin compositions impacting the density and porosity of the thrombus [130]. ILT is on the other hand a highly important indicator of AAA. The altered thrombus composition of the thrombus might incur expansion protection [119].
- Additional transforming growth factor β (TGF β) signals [131].

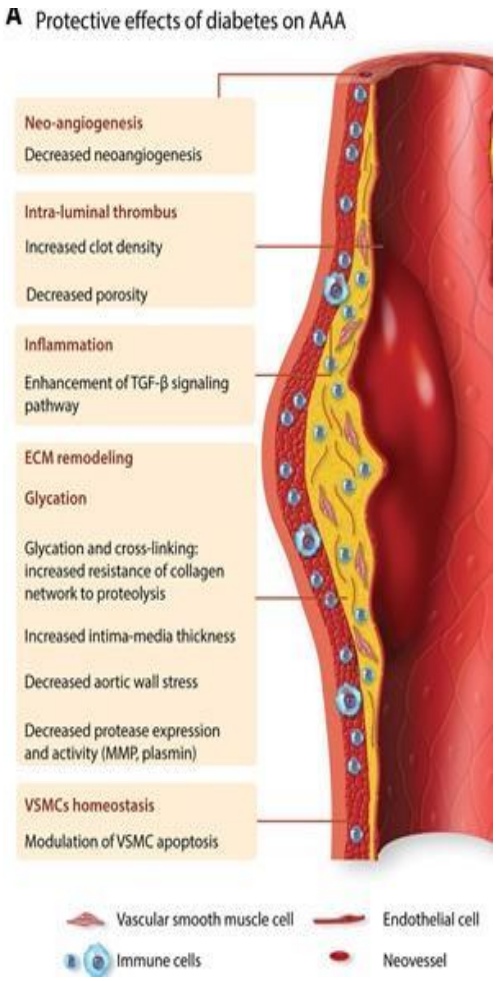

Figure 21. Mechanisms for diabetes-induced protective effects against AAA.
Reproduced from [119] with permission from Oxford University Press.

Overall aims

Relationships between glycemia and aortic stiffness (paper I)

To investigate the association between glucose metabolism, diabetes, and arterial stiffness in two related generations.

Relationships between AAA and biomarkers (paper II)

To investigate the association between biomarkers and AAA after long-term follow-up.

Relationships between glycemia and AAA (papers III - V)

To investigate associations between glucose metabolism and diabetes with aortic dilatation and AAA from three different perspectives:

- Aortic dilatation at the initial stages of diabetes.
- Effects of diabetes during long-term follow-up after elective EVAR.
- Effects of diabetes during long-term follow-up after acute AAA repair.

Methods

Measurement techniques

Blood pressure and lipids (papers I - III)

Systolic and diastolic blood pressures (SBP, DBP) were measured (mmHg) on subjects´ non-dominant arm with an Omron device (Omron, United States) after five minutes rest, as the average of two measurements with one-minute intervals.

Plasma (p-) triglycerides, low density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C) cholesterol levels were obtained utilizing a Cobas© device (Roche, USA). Weight (kg) and height (m) were measured in light indoor clothing and body mass index (BMI) calculated as kg/m^2.

Aortic stiffness (paper I)

Each subject's cfPWV was measured using arterial tonometry as the median of two measurements (three in case of large variations) in the supine position with a Sphygmocor© device (Atcor, Australia). Subjects rested for five minutes before the first measurement and then one minute between the first and the second sample. The pulses were measured at the carotid artery and the femoral artery, respectively, and the distance between the two points multiplied by 0.8. The pulse transit time from carotid to femoral was divided by the length in between to estimate PWV.

This *regional* method gives the value as an average of several vessels rather than a direct measurement of the pulse speed at a specific location. It also suffers from the need to apply contact probes at exact locations and monitor the pulse. There are new local less invasive non-contact measurement techniques underway [132] but the carotid-femoral technique using a Sphygmocor© device is considered as a state of the art (golden standard) technique [133].

Glucose metabolism (paper I) and diabetes (papers I, III - V)

FPG was analyzed via biochromatic analysis of light absorbance of a blood sample after the reaction with adenosine triphosphate (ATP) (Cobas©, Roche, USA). The amount of reduced nicotinamide adenine dinucleotide phosphate (NADPH) was proportional to the fasting glucose concentration.

In papers I and III, diabetes was self-reported or registered in the local database for newly detected DM patients in Region Skåne, ANDIS. In papers IV and V, the Swedish National Diabetes Register (NDR) was used to identify subjects with a DM diagnosis.

Aortic dilatation and AAA (papers II-III)

Ultrasound screening for AAA was performed by biomedical scientists and registered vascular nurses using the LOGIC® ultrasound machine and 3.5–12 MHz probes (General Electric Healthcare Inc, Chalfont St. Giles, UK). In patients with inconclusive US results, a CT scan without contrast was performed to measure the diameter of the aorta. The widest diameter of the infrarenal aorta was measured leading edge to leading edge with calipers on the outer layer of the anterior wall and the inner layer of the posterior wall, a diameter of ≥30 mm defines an AAA.

Biomarkers (paper II)

Immunoassays are considered to be the state of the art for detection of low concentrations of biomarker proteins [134]. This method is based on mixing a sample of tissue or blood with a solution where the corresponding antibody for the protein under consideration is present and will bind to the protein under consideration [135]. Figure 22 summarizes the process of inserting an antibody which binds to the antigen and leads to an optical signal in the instrument.

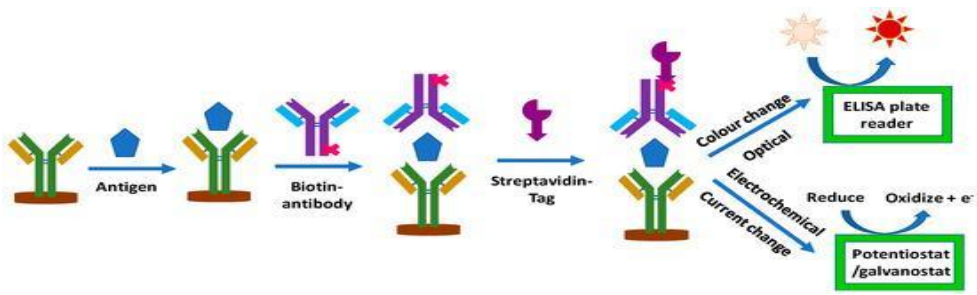

Figure 22. Principles of ELISA immunoassay for protein detection.
Reproduced from [136] with permission from MDPI.

As mentioned above, there is a vast number of proteins in human cells and blood vessels with potential links to AAA formation. As we used data from the Malmö Diet Cancer Study (MDCS) screening, the selection of markers was based on what had been measured at baseline, or analysis of thawed samples used for predominantly cardiovascular biomarker research in the screening cohort rather than what would be assumed to correlate the most with aortic dilatation or aneurysm. Methods for measurement of the different biomarkers analyzed in paper II are summarized in Table 4 below.

Table 4. Biomarker detection methods

Biomarker	Technique	Interassay coefficent of variation (%)
Copeptin	Chemiluminescence assay	4.6
C-reactive protein	High sensitivity assay	4.6
Cystatin C	Particle-enhanced immunonephelometric assay	4.3
Proneurotensin	Chemiluminometric sandwich immunoassay	8
Lp-PLA 2	Immunonephelometric assay	5.8
MR-proANP, MR-proADM	Immunoluminometric sandwich assay	10

The interassay coefficient is a measure of the reliability of results, stating the coefficient of variation (standard deviation/mean) for results from repeated measures of the same sample [137]. A value below 15 % is considered adequate and 5 % is considered as highly reliable [138].

Materials, data sources

Population studies from Malmö (papers I-III)

- Malmö Diet and Cancer Study, MDCS

MDCS ran from 1991 through 1996 at the Department of Clinical Sciences, Lund University and Skåne University Hospital in Malmö. The inclusion criterion was to be a resident of Malmö born between the years of 1923 and 1950, corresponding to ages between 44 and 73 years at baseline. Participants were invited through mail and public announcements [139]. The initial screening had the aim of exploring relationships between intake of different nutrients and cancer [140].

In total, 28098 individuals attended the procedure with a combination of measurements, a self-reporting questionnaire and a multi-day diary of food consumption. A subset of the participants in the MDCS were invited to take part in the Cardiovascular arm (MDCS-CV) with a special focus on heart-related conditions. 6103 subjects volunteered for measurements and questions related to arterial stiffness and glucose metabolism [141]. All living participants were invited to a reexamination during the follow up period 2007 – 2012, where the procedure was repeated. Data from the MDCS-CV were used in paper II, where values of several biomarkers in the initial screening were related to aortic diameter and aneurysm incidence at subsequent US screening for AAA.

- Malmö Offspring Study, (MOS) (paper I)

The MOS cohort [142] was used in paper I to assess relationship between FPG, HbA_{1c}, and SAF in relation to carotid-femoral PWV. In MOS, children and grandchildren of 6000 of the original MDCS participants were invited to take part in a survey of data like the one offered to the initial CV cohort, i.e. with diet reporting and measurements of:

- Blood pressure, arterial stiffness, spirometry
- Self-reported lifestyle, socioeconomic factors, medication
- Lipid profile, BMI, glycemic control

Ultrasound (US) AAA screening (papers II-III)

As mentioned, all Swedish men are invited for US screening of their infrarenal abdominal aortic diameter during the year of their 65^{th} birthday [40]. The Skåne Region joined in 2010 and it has been estimated that 78 % of male citizens in the Malmö area chose to participate [48]. Results obtained through the AAA screening examinations in Malmö [42] were used in:

Study II, to relate biomarker levels obtained at the previous MDCS baseline examination to aortic diameter and aneurysms at screening 14-19 years later.

Study III, to evaluate if aortic diameter in 65-year-old men recently diagnosed with DM differed from that in 65-year-old men without DM.

Population registers

- ANDIS (paper III)

Rapidly rising rates of DM in Sweden's southernmost region Skåne motivated the creation of a local database ANDIS (All new diabetic patients in Skåne) in 2008 [143]. ANDIS was used in paper III.

- National Diabetes Registry (NDR, papers IV-V)

The NDR was set up in 1996 and gathers records for DM cases in all of Sweden [144]. NDR registrations were used in papers IV and V.

- Swedish Vascular Registry (Swedvasc, papers IV-V)

Swedvasc is a validated data base containing records of surgical procedures to arteries and veins other than in the heart or brain performed at Swedish hospitals since 1994 [145]. This register was used in studies IV and V.

- National Board of Health and Welfare Registers (papers IV-V)

The National Board of Health and Welfare maintains many registries from the Swedish healthcare system. A number of these were used to supply data for the purpose of papers IV and V:

- Cause of Death Register: All fatalities in Sweden [146].
- Inpatient Registry: All completed visits (hospitalisations) to specialist, emergency and psychiatric care [147].
- National Cancer Registry: All cases of cancer in Sweden, subdivided by region [148].
- Prescribed Drug Register (from 2005): All purchases of prescription pharmaceutics in Sweden [149].

National Baseline Data

- Longitudinell integrationsdatabas för sjukförsäkrings- och arbetsmarknadsstudier (LISA, papers IV-V)

Background data on employment, salaries, residency, nationality and other socioeconomic factors falls under the responsibility of Statistics Sweden (SCB) [150]. This authority´s integrated database for labor market research (LISA) was used in papers IV and V.

Ethics

Ethical approval has been provided for all papers as follows:

Paper I: MOS was approved by the Regional Ethics Committee in Lund (Dnr. 2012/594).

Paper II: MDCS study protocols were approved by the Regional Ethics Committee in Lund (Dnr. 2009/633 and 2013/566).

Paper III: Study approved by the Regional Ethics Committee in Lund (Dnr. 2016/232).

Papers IV-V: Studies approved by the Regional Ethics Committee in Lund (Dnr. 2016/232 and 2016/544).

Statistical Techniques

Cohorts and sample sizes

All the presented papers were cohort studies, population-based or patient-based. Determination of sample sizes is a tradeoff between available resources and certainty, and the number of study participants should be carefully planned [151 - 152]. Table 5 summarizes the cohort inclusion criteria and sample sizes used.

Table 5. Cohorts and sample sizes in the presented papers.

Papers	Cohort	Sample size (n)
I	Descendants of MDCS participants	2640
II	Participants in both MDCS-CV and ultrasound screening for AAA	117
III	65-year old men with and without newly diagnosed DM	18953
IV	Patients having undergone elective EVAR for AAA	3378
V	Patients having undergone acute AAA repair	2217

Spearman rank correlation (paper II)

The correlation coefficient is normalized and falls between -1 and +1. A value above 0.4 indicates a moderate positive correlation between the variables, and anything above 0.7 is considered strong. Analogously, anything between -0.7 and -1 means a strong negative relationship [153]. Spearman rank analysis is a special form of correlation that uses *ranks* rather than actual test values. Each value in both the input vector X and output vector Y is assigned a number corresponding to its rank, i.e. its position in a sorted list with all the observations. The calculations are similar to the ones for Pearson correlation, except that these ranks are used rather than the observed values.

Linear regression (papers I-II)

Linear regression seeks to quantify the relationship between two or more phenomena measured as numerical values. It attempts to find a best possible linear function based on the set of *observations*, i.e. pairs of predictor and response measurements. Regression works best when both predictor and response variables adhere to a normal distribution. If this is not the case, one may achieve a less skewed distribution by applying the natural logarithm to the original values. Another frequently used transformation is to subsequently standardize each value, calculating the log Z-score.

Logistic regression (paper III)

Logistic regression is used when both predictor and response are numerical, logistic regression is applied when the independent variable/s are measured on a scale and the outcome is dichotomous. It seeks to determine how the probability of a certain response changes based on the value of the predictors.

Propensity score analysis (papers IV-V)

The outcomes under consideration in papers IV and V were death rates and incidence of various cardiovascular diseases, and we wanted to determine the effect size of DM2 vs. no DM2. It should be expected that many other background values could alter these results, most notably smoking and certain pharmaceutics [154] but also hypertension and high cholesterol [155], as well as socio-economic background [48]. The task concerns how to isolate the studied relationship in the generated data.

For paper I, we made use of regression techniques built into SPSS multiple regression analysis, which can be used for problems with collinearity when using a limited number of predictors [156]. However, the survival based analysis performed in papers IV and V and the inclusion of a vastly greater number of factors from socio-economic registers led to a need for a more extended computational method.

A propensity score is the conditional probability of being exposed to a certain outcome, for example, aortic re-interventions, given a set of values representing the individual's covariates. This can be used to adjust results and isolate the desired effect, finding the associated probability for the phenomenon under consideration [157]. One would then look at how these scores overlap for two cohorts.

Student's t-test comparing means (papers I, III)

One of the most ubiquitous usages of hypothesis testing is the comparison of means between two groups with a *t-test*. Samples from both categories are drawn, a formal test procedure carried out to determine whether results are significant or not.

Chi square test (paper III)

The chi-square test is used in a situation where:

- There is only response variable and only one predictor
- Both are categorical variables

The chi-square test determines whether there is an association between the two variables.

Survival curves (papers IV - V)

A *survival curve* shows how many of the original population are still “alive”, i.e. did not yet experience the event under consideration. This is displayed as a graph showing the proportion of individuals at different points in time after the starting point. When comparing two groups, one often quantifies the comparison of death rates in group A relative to group B as the risk ratio (RR).

Methods, results and conclusions

Paper I

Theory: Diabetes and other stages of impaired glucose metabolism lead to increased arterial stiffness (Figure 20). However, most studies did not examine whether the association is of the same magnitude across age groups in adult life. While it has been shown that higher age is a strong determinant of stiffer arteries (Table 1), it has to the author's knowledge not been examined whether people have the same sensitivity for arterial stiffening caused by increasing blood glucose concentrations depending on their age.

Data: Collected from N=2,640 subjects from the MOS divided into parents (children of MDCS participants) and children (grandchildren of MDCS participants) as shown in Figure 24.

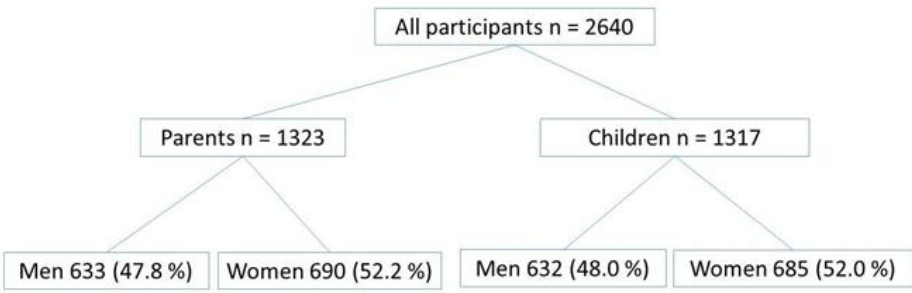

Figure 24. MOS participants in two generations used for Paper I.

Method: Multiple regression models where the dependent variable was the natural logarithm of PWV. A number of quantities were used as predictors, most importantly Z-scores of various measures of glucose metabolism, i.e. FPG, HbA1c, and SAF. For confounder adjustment purposes, the multivariate model also included age, sex, triglycerides, LDL and HDL cholesterol, The analysis was divided into two groups, one for parents (children of MDCS-CV participants) and one for children (grandchildren of MDCS-CV participants).

A t test was also used to check if pulse wave velocities were different for people with diabetes compared to those without, once again separately for each generation.

Results: HbA_{1c} and FPG were positively associated with PWV, but only in the older generation (children of MDCS-CV participants). Among children (grandchildren of MDCS-CV participant), the regression coefficients were not significant. SAF was not significantly related to PWV in either of the groups.

Table 6. Regression coeffiencents for paper I. FPG, HbA_{1c} and SAF transformed with natural logarithm and standardized to Z-score, Pulse Wave Velocity transformed with natural logarithm.

Measurement	All		Parents		Children	
	Beta	P-value	Beta	p-value	Beta	p-value
FPG	0.007	0.018	0.013	0.008	0.002	0.679
HbA_{1c}	0.017	<0.001	0.022	<0.001	0.009	0.170
SAF	0.001	0.767	0.008	0.208	-0.006	0.227

Mean pulse speed differed between participants with and without diabetes in the total group (mean difference 1.7 m/s; $P<0.001$), as well as within each generation (parents: 1.3 m/s; $P<0.001$, and children: 0.7 m/s; $P=0.040$).

Conclusions: Increased fasting plasma glucose concentrations and HbA_{1c} levels were associated with increased aortic stiffness, but only in the older cohort.

Paper II

Theory: Concentrations of certain proteins have been shown to correlate with aortic diameter and prevalence of AAA. While this shows that they can be used as biomarkers of an existing or later developed aneurysm, it could be of interest if their levels have an association (prediction) also with future aortic growth. Such links would enable the screening and detection of individuals at risk for AAA in advance.

Data: A total of 117 subjects could be identified from their participation both in MDCS CV between 1991 and 1996 and subsequently in the US screening for AAA in 2010. Given that smoking has been proven to be an important determinant of aortic diameter, they were stratified according to whether they currently smoke or had a history of smoking. The division is displayed in Figure 25.

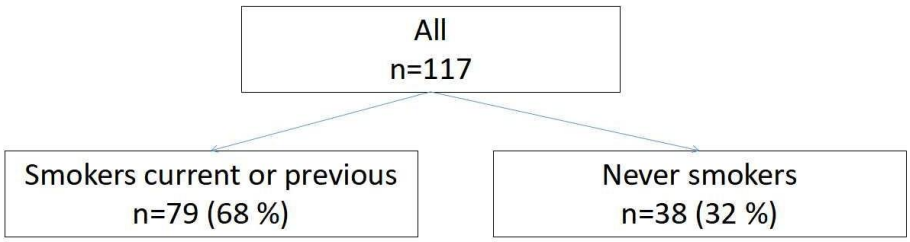

Figure 25. Paper II subjects classified by smoking status.

Method: Spearman rank correlations and linear regressions coefficients (beta) were estimated for the association between a number of biomarkers (presented in Table 7) and aortic diameter measured 14-19 years subsequently. The regression model was adjusted for age and smoking.

Results: Separate correlation constants were calculated between levels of each of the seven measured biomarkers (CRP, PNT, CPT, Lp-PLA2, Cyst C, MR-proANP, and MR-proADM) measured at baseline and the aortic diameter in 2010.

Table 7. Correlations between aortic dilatation and biomarker concentrations for a number of proteins.

Biomarker	Spearman r	p value
CRP	.153	.108
PNT	.070	.469
CPT	.156	.101
Lp-PLA2	.024	.798
Cyst C	.015	.877
MR-proANP	.014	.880
MR-proADM	.117	.218

None of the correlations were significant.

Conclusions: The tested biomarkers could not be used to indicate aortic dilatation later in life.

Paper III

Theory: DM2 has been shown to be negatively associated with AAA growth and aortic dilatation. Most other studies did not consider the length of time from DM2 diagnosis to AAA diagnosis, but this study was set up to examine if the apparent protective effects of hyperglycemia are present also during the earlier stages of a diabetes process.

Data: The original source was the results from US screening of aortic diameter including a total of 20,363 65-year old men performed from 2010 to 2016. These individuals were crosschecked against ANDIS and classified into two groups; a) With DM2 recently diagnosed between 2008 and 2015, and b) without DM2. Subjects with a DM2 diagnosis earlier than 2008 were excluded from comparison, as indicated in Figure 26.

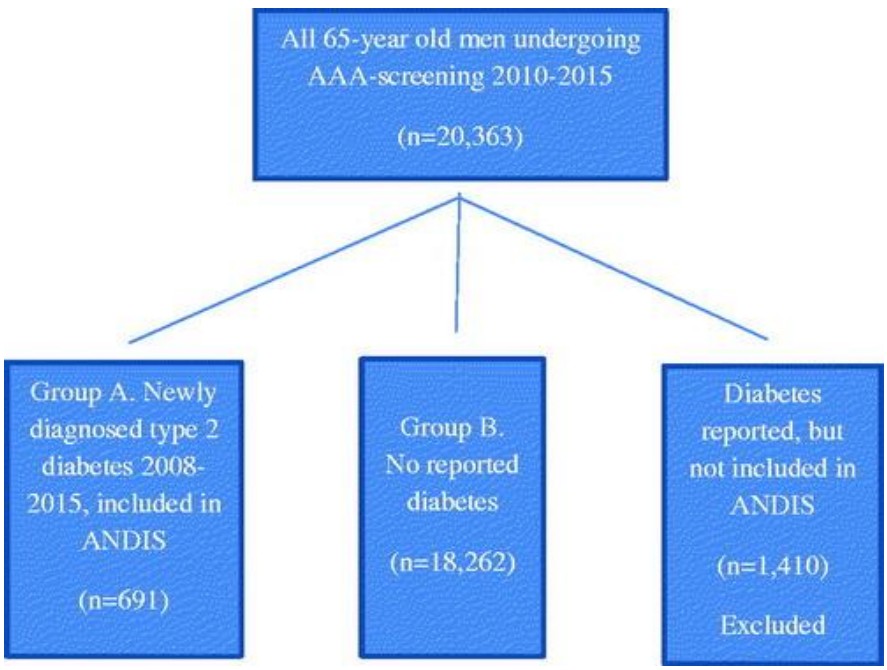

Figure 26. Paper III cohorts of men undergoing AAA screening.

Methods: The basis of the conclusions in paper III was a t test for the mean of aortic diameter in 65-year-old men with DM vs. 65-year-old men without DM. A Logistic regression model was used to estimate which factors had a significant impact on the risk of having AAA. Chi-squared tests were used to determine if the prevalence rate of aneurysm or aneurysm in formation differed between DM and non-DM groups.

Results: A t-test comparing aortic diameter for participating men with a recently diagnosed or incipient DM to those without diabetes did not yield a significant difference. The DM cohort had a median of 18.8 [17.4 – 20.8] mm compared to a median of 19.0 [17.5 – 28.7] mm for men without DM. Table 8 compares the results for the two groups.

Table 8. Comparison DM – non DM. Median (Interquartile range (IQR)) or n (%).

	Group A DM (n=691)	**Group B, non-DM (n=18,262)**	**P-value**
Aortic diameter (mm)	18.8 (17.4-20.8)	19.0 (17.5-20.5)	0.427
Aneurysm in formation (25-29 mm)	20 (2.9)	473 (2.6)	0.17
AAA (≥30 mm)	17 (2.5)	282 (1.5)	0.010
Large AAA (≥50 mm)	0 (0.0)	34 (0.2)	0.626

AAA prevalence was significantly higher in men with newly detected DM, but this difference did not persist when confounders (BMI, smoking, atherosclerotic disease, and hypertension) were considered. Among men with newly detected DM, C-peptide correlated significantly with aortic diameter (p=0.034), whereas HbA_{1c} did not (p=0.243).

Conclusions: The previously found negative link between DM2 and AAA could not be identified among subjects with a recently acquired diabetes diagnosis.

Paper IV

Theory: It has been noticed that impaired glucose metabolism is associated with lower rates of AAA and aortic dilatation. Do the proposed protective effects of DM2 against AAA development also lead to lower risks of mortality and re-intervention during the follow-up after elective EVAR?

Data: All patients undergoing elective AAA surgery in Sweden between 2009 and 2015. They were then further selected to only include those having been treated with EVAR (N=3,378). They were cross-checked against the NDR for DM2 as indicated in Figure 27.

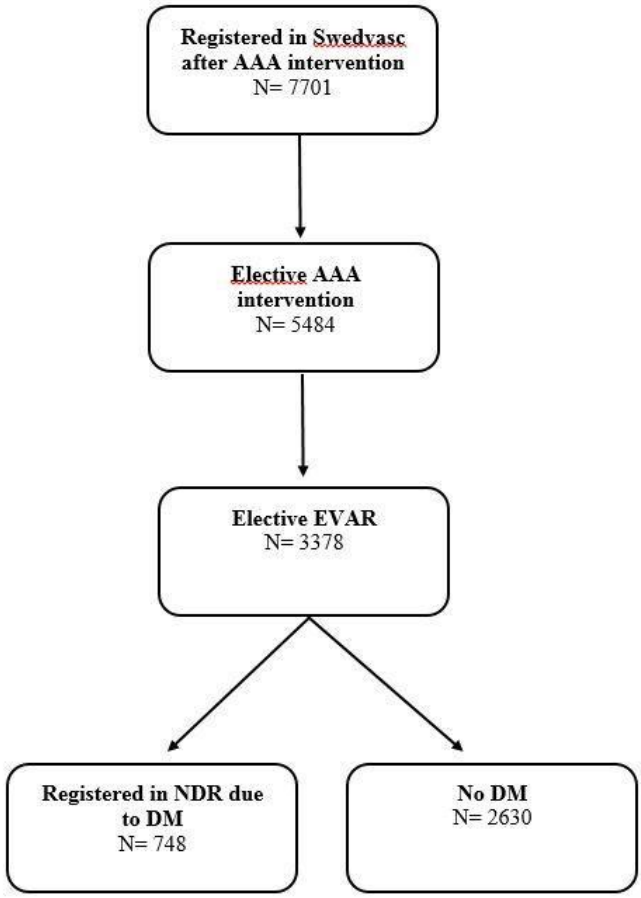

Figure 27. Paper IV subjects.

Methods: Survival analysis investigating the outcome after elective EVAR surgery, calculating several endpoints:

- Mortality
- Cardiovascular mortality
- Re-intervention rate
- AMI
- Stroke
- Major adverse cardiovascular events (MACE)

Adjustment was performed using propensity score analysis for several variables with confounder potential:

- Background socioeconomic factors: age, sex, education, smoking, income, civil status, and country of birth.
- Medications: lipid-and blood pressure lowering drugs, aspirin, anticoagulation, ACE-inhibitors, angiotensin receptor blockers, alpha-, beta-, and calcium channel blockers, diuretics, or digoxin.
- Medical history: Previous AMI, coronary heart disease, stroke, cerebrovascular disease, atrial fibrillation, congestive heart failure, renal disease, malignant disease, liver disorders, psychiatric disorders, peripheral arterial disease, or chronic obstructive pulmonary disease.

Results were compared between patients with and without DM.

Results: DM patients had lower need for re-intervention after elective EVAR than subjects without diabetes, whereas there were no significant differences in cardiovascular mortality or overall mortality between groups. Figure 28 compares unadjusted cumulative incidences of mortality, cardiovascular diseases, and re-interventions in patients with DM (blue lines) to those with no reported diabetes (red).

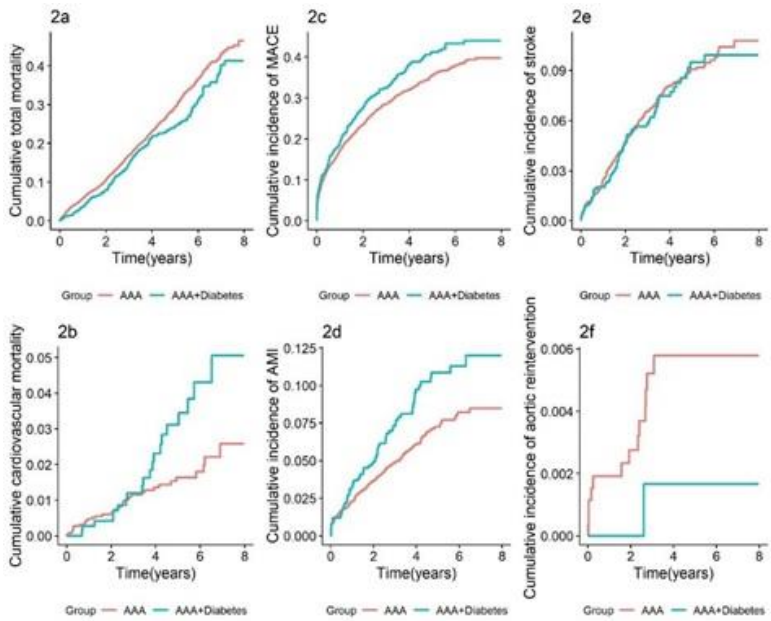

Figure 28. Kaplan-Meier curves for mortality and cardiovascular outcomes in paper IV. Unadjusted cumulative incidence of 2a) mortality, 2b) CV mortality, 2c) MACE, 2d) AMI, e) Stroke and 2f) Re-intervention.

Table 9 shows estimates of hazard ratios (HR) for the various endpoints including mortality, MACE, AMI, stroke and aortic re-intervention among patients with diabetes after adjustment for confounders.

Table 9. Hazard ratios (HR) for patients with DM –compared to those without -DM for the studied outcomes.

Endpoint	HR	95% CI	p-value
Mortality	0.88	0.74-1.05	0.1470
CV mortality	1.58	0.87-2.86	0.1336
MACE	1.09	0.94-1.27	0.2380
AMI	1.44	1.06-1.95	0.0201
Stroke	0.95	0.68-1.32	0.7513
Aortic re-intervention	0.12	0.02-0.91	0.0406

DM patients had higher risk for AMI but lower risk for aortic re-intervention.

Conclusions: DM patients had lower need for re-interventions and higher risk for AMI than patients without DM, whereas mortality did not differ between groups. As diabetes by itself is expected to incur higher risks of death, a protective effect from DM2 in AAA patients after follow-up from elective EVAR cannot be excluded.

Paper V

Theory: Paper IV indicated no increased mortality in diabetic patients after elective EVAR surgery. Do such relationships exist during long-term follow-up after acute AAA repair?

Data: All individuals registered in Swedvasc for acute AAA surgery between 2009 and 2015 (N=7,701). They were subsequently divided into subgroups with and without a diagnosis of diabetes registered in the NDR.

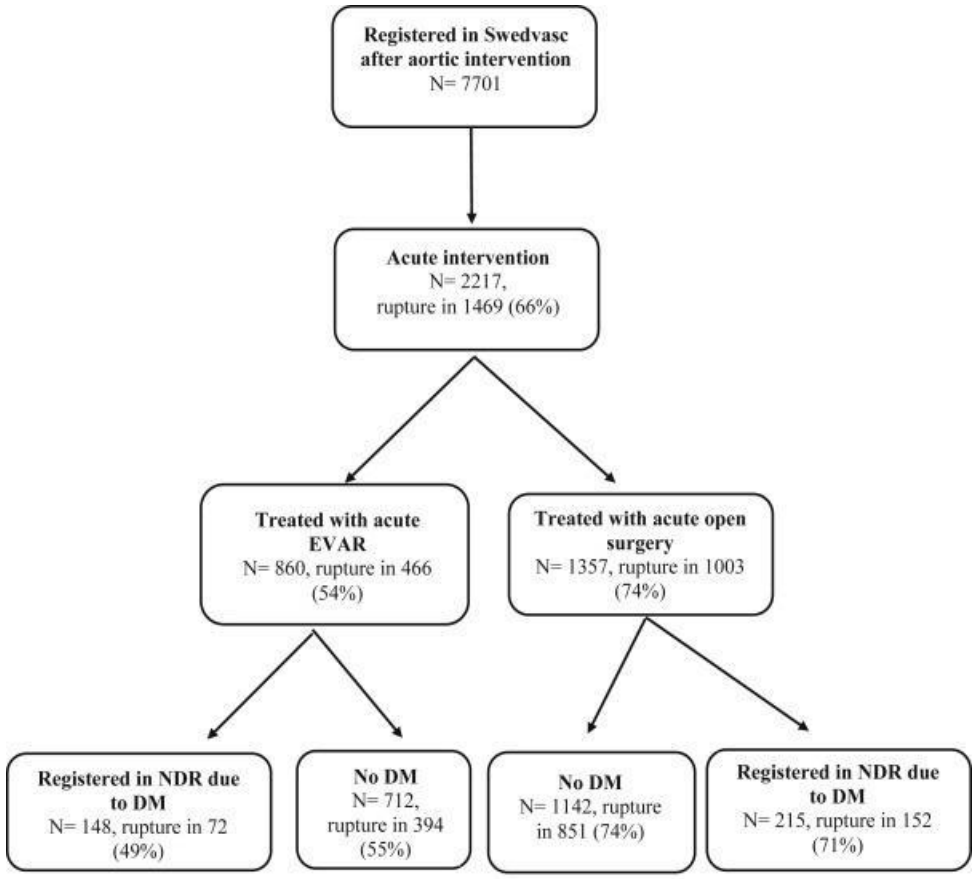

Figure 29. Paper V Cohorts.

Method: Survival analysis including Kaplan-Meier curves investigating the outcome after acute aortic repair, calculating the respective prevalence rates of the same outcomes as in paper IV. Results were compared between DM patients and non-DM subjects by propensity score adjusted analysis. The calculation of quantified differences in risk between subjects with and without DM was performed following adjustment for the same explanatory factors as in paper IV above, with the important additions of adjustment for surgical technique (EVAR or open surgery), and for the preoperative occurrence of aneurysm rupture or not.

Results: During long-term follow-up after acute aortic repair, patients with diabetes experienced a lower rate of cardiovascular and total mortality than those without DM. Rates of MACE, AMI, and stroke did not differ between the cohorts. Figure 30 visualizes unadjusted cumulative incidences of several conditions, comparing patients with DM (blue lines) to those without reported diabetes (red).

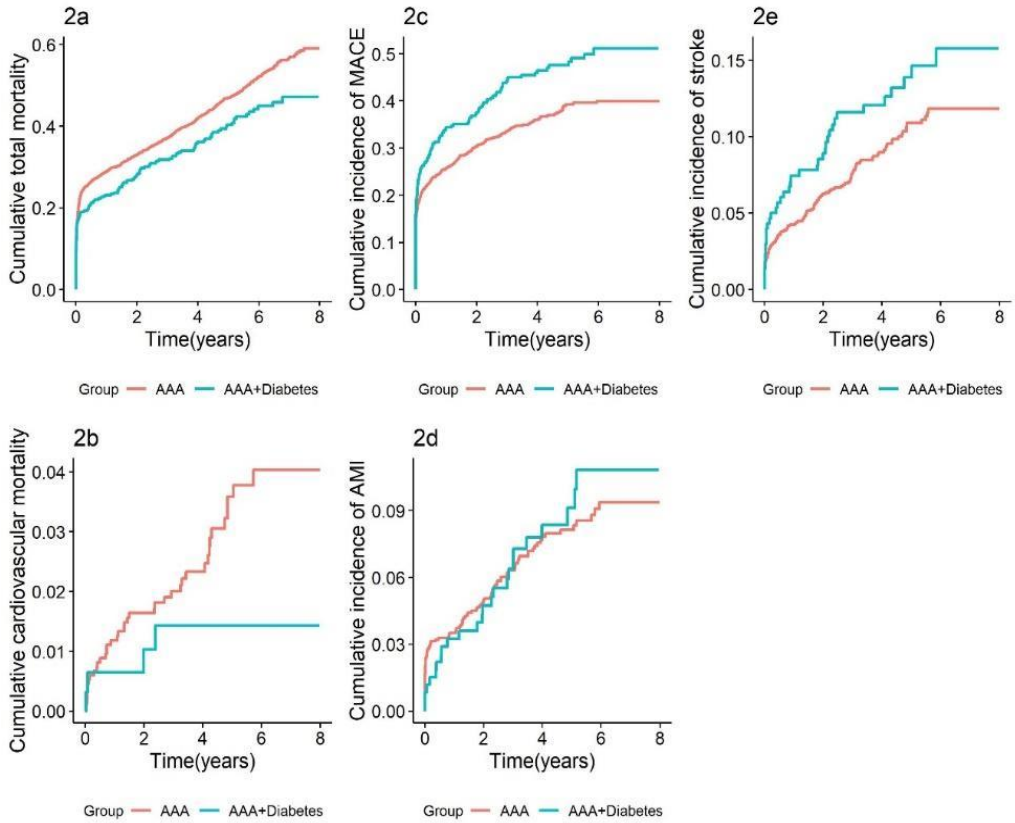

Figure 30. Kaplan-Meier curves for outcomes in paper V.
Cumulative incidence of 2a) mortality, 2b) CV mortality, 2c) MACE, 2d) AMI, 2e) Stroke.

Adjustment was performed by propensity score analysis adjusted as in paper IV (outlined on page 36) with the addition of adjustment for surgical method (EVAR or open surgery), and for rupture or not. Table 10 quantifies HR estimates between DM2 and non DM2 subjects after adjustment.

Table 10. Hazard Risk ratios DM – non-DM for the studied outcomes, all values adjusted for confounders.

Endpoint	HR	p-value	95% CI
Mortality	0.75	0.0156	0.59-0.95
Cardiovascular death	0.17	0.0013	0.06-0.50
MACE	1.10	0.4235	0.87-1.41
Acute myocardial infarction	1.36	0.3667	0.70-2.77
Stroke	1.31	0.2331	0.84-2.03

The DM2 group had significantly lower risks of total mortality and cardiovascular death than non-DM subjects.

Conclusions: Following acute AAA surgery, DM2 patients had lower cardiovascular and total mortality than non-DM2 subjects.

Discussion

Subject selection, validity of data

A study sample should normally be representative of the population in terms of health and socio-economic characteristics. There are several forms of bias, which are most easily avoided by randomized sampling methods [158]. In screening settings, non-response bias, i.e. the possible difference between participants and non-participants has also to be considered as participants are often reported to be healthier than non-participants [159- 160].

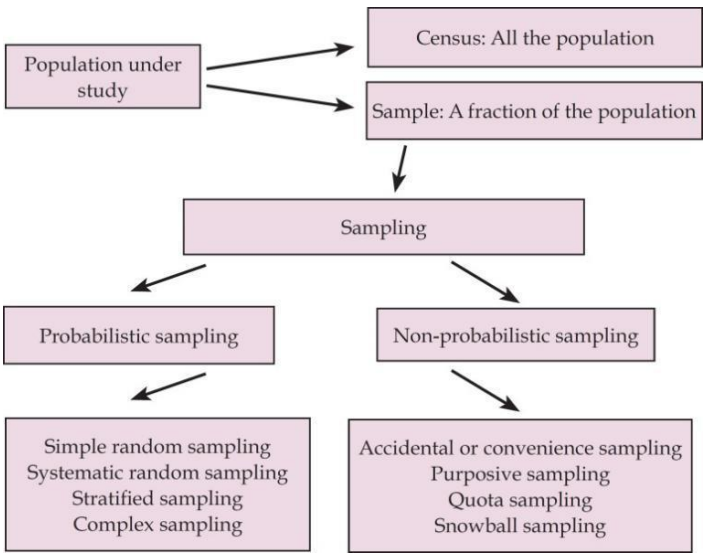

Figure 31. Sampling techniques to avoid bias.
Reproduced with permission from [161] from Scielo Analytics.

In papers I-III, conclusions are derived from research based on voluntary participation. As population-based cohorts were invited, there is no sampling bias, but those who complied with the invitations and were included were most likely in better health than those who did not participate [48]. The general conclusions drawn

regarding associations between different conditions might therefore not perfectly reflect associations in the entire population.

In the original MDCS study, a total of 71,104 men and women between the ages 43 and 70 years were invited, representing the entire age group, but only a total of 30,000 underwent all examination procedures [162]. The loss of more than half of the theoretical highest possible sample size was due to several reasons:

- Death
- Relocation
- Refusal
- Lack of literacy of Swedish text
- Incomplete questionnaires
- No answer or unknown address

A subsequent follow-up on both participants and non-participants revealed that the volunteers had lower rate of mortality and better health than absentees [163].

From the MDCS participants, a random sample of 50 % (every other person) received an invitation to undergo a set of extended measurements; the cardiovascular arm (MDCS-CV). Of the 14,000 invited individuals, a total of 6,103 subjects chose to take part also in this set of measurements [164]. No specific analysis has been done as to whether those choosing to volunteer for the second part had other health characteristics than the average of MDCS participants.

The MOS cohort consists of all living descendants (children and grandchildren) to every participant in the MDCS-CV [142, 165]. Official birth data has shown that 15,000 persons in all can be included, and it is expected that a total of 5,000 – 6,000 will participate during 2013 to 2021.

Paper III is based on data on diabetes collected through ANDIS, the regional database for newly detected DM2 cases in Skåne. Primary health care centers and hospitals are instructed to add all new cases to the database, and it has been estimated that more than 90 % of eligible cases are reported [166]. No bias with regards to AAA development can be expected among the missing subjects.

All 65-year old Swedish men are invited to ultrasound screening, and average attendance between 2006 and 2014 was 84 % in on a national level [46] and 78 % in Malmö [48]. There is no sampling bias as the entire male population of that age is invited, but it is presumable that a “healthy volunteer bias” exists [48]. It is important to note, however, that the screening is recommended by the Swedish Board of Health and Welfare [167] and motivated by health concerns and not for recruitment of volunteers for scientific studies.

Papers IV-V are based strictly on national register data. There is no sampling bias as all Swedish patients undergoing AAA surgery during a certain time period were included, and no volunteering bias as participants were selected from data bases. One may consider whether people undergoing AAA surgery differ from the rest of the population with regards to the link between DM2 and AAA. Furthermore, one should keep in mind that it was never analyzed to which extent individuals with DM2 differed from those without before surgery.

Glycaemia and aortic stiffness

The link between elevated glucose concentrations and indicators of arterial stiffness was confirmed in paper I. However, it was observed that this association was only significant in the older generation. Apparently, younger people are less sensitive to arterial stiffness induced by hyperglycemia. The more advanced vascular ageing process in the elderly parental generation might have made their arteries more sensitive to increasing hyperglycemia causing more pronounced stiffening, whereas such a relationship between impaired glucose metabolism and higher cfPWV values was not yet detectable in younger individuals. It could be of interest to repeat the analysis based on strata with different PWV ranges to evaluate whether younger people with above average stiffness have the same link to hyperglycemia as older subjects in a similar condition, or if age is the determining factor. As none of the pathways outlined in Figure 20 were evaluated in our study, any theories on which of the mechanisms are more present for older people remains speculative. Overall, the younger group seems to be less sensitive (in theory) to the listed possible mechanisms for restructuring of the aortic wall. The fact that individuals with a DM diagnosis had significantly higher mean cfPWV than those without was expected and a confirmation of previous findings [168-169].

Biomarkers and aortic dilatation/aneurysm

The measured levels of several biomarkers did not correlate significantly with aortic diameter and AAA occurrence at screening after the 14-19 years of follow-up reported in paper II. Based on these results only, it seems to be of limited value to use the selected studied selected protein levels as predictors of future development of aneurysms.

As these findings are partly in contrast to previously reported cross-sectional relationships between aortic diameter at ultrasound screening for AAA and protein markers [170], the long time-span between measurement of biomarkers and

assessment of aortic diameter by US screening for AAA in this study must be kept in mind. In the MDCS both Lp-PLA2 activity and mass [171] and MR-proADM [172] were markers of future AAA hospitalization during long term follow-up, however, supporting the notion that AAA is an athero-thrombotic related disease related to long-standing cardiovascular stress on the aortic wall. It appears that the studied plasma biomarkers might either not be relevant until closer to AAA diagnosis, or only for prediction of large AAA requiring hospitalization and surgery. Different markers might perhaps be relevant in different steps in aortic dilatation and AAA formation [173]? The absence of relationships in the present study might also be attributed to a type 2 statistical error given the small sample size. The results should therefore in no way exclude the potential of biomarkers in future methods for AAA diagnosis.

Glycaemia and aortic dilatation/aneurysm

The main theme of the thesis, the relationship between glucose metabolism and abdominal aortic aneurysm was studied in papers III – V:

- In the early stages of diabetes, there are no significant differences between DM and non-DM individuals in terms of aneurysm development and aortic dimensions. It should be noted that only cases of recently diagnosed DM were included in the study, and that the timeline of US measurement and DM2 diagnosis differed greatly among the participants. DM is in most patients a slow process and might take years to evolve [174].
- The lower frequency of re-interventions after EVAR and lower mortality after acute surgery among the DM2 cohorts suggest a potential protective effect against the hazardous risks during follow up after AAA repair.
- The influence of pharmaceutics for conditions other than diabetes was adjusted for in papers IV-V. It must be taken into account that the use of commonly used [175] Metformin and other antidiabetic drugs were not adjusted for. There are indications of a beneficial effect of Metformin intake on AAA [176], and the concept is currently being tested [177]. Metformin effects could therefore not be excluded in the explanation for DM patients lower mortality in DM patients.
- It is worth speculating whether all cases of DM2 and AAA are recorded in registers, but there is no apparent reason to believe that hospital staff have been violating guidelines or committed clerical errors in more than possibly a few cases. The influence of other factors has been accounted for with adjustment for a variety of confounders.

- As discussed, AAA is much more prevalent among men compared to women. Whereas it should be noted that conclusions in paper III were based on only male participants, papers IV and paper V both had sizeable numbers of female patients. Sex was adjusted for in the propensity score analysis, but it was not evaluated whether the same patterns were occurring in both genders.
- The number one determinant of higher risk for AAA and its complications is cigarette smoking [41-42]. Hence it is of utmost importance to account for the influence of smoking habits in the calculations, isolating the link between DM2 and aneurysms. Information on the smoking status of each participant was collected and used for the confounder adjustment process in all presented papers.

The interpretation of these statistical results must be that there is a physiological pathway linking impaired glucose metabolism and protection against AAA. As all studies have been performed on an epidemiological basis, there is not enough ground for speculation on what cardiovascular processes constitute the underlying causes for the observed associations. Besides the mentioned possible mechanisms in Figure 21 it seems worthwhile to speculate if the added stiffening of the arteries observed in the diabetic state could make them less prone to aneurysm development. However, such associations are not evident as other studies found either no link [178] or an opposite positive correlation between stiffness and aneurysms [179]. These studies were focused directly on the correlation between stiffness and aneurysms, and this was not considered in the presented papers.

Blood vessel function in the hyperglycemic state

As mentioned, all five papers were strictly epidemiological. All screenings used statistical methods to establish potential associations on a macroscopic scale between various measured physiological mechanisms. In depth microscopic examinations of cells and/or arteries were not performed. Different pathophysiological processes in the different sections of the aorta seem to be the unifying mechanism. The excess amounts of glucose present in the blood stream induces modifications and imbalances for several of the components of the aortic intima, media and adventitia. The individual effects of each of these diabetes-related mechanisms are shown in Table 11.

Table 11. Pathways Diabetes – Aortic stiffness – AAA. ECM = extracellular matrix, AGE = advanced glycation end products.

Diabetes-related process	Association with arterial stiffness	Association with aortic dilatation
Increased carotid intima media thickness (CIMT)	Positive [180]	Negative [181]
ECM glycation and formation of AGEs	Positive [182]	Negative [183]
Oxidative stress	Positive [184]	Positive [185]
Endothelial dysfunction	Positive [186]	Positive [187]
Decreased Angiogenesis	Unclear	Negative [188]
Calcification	Positive [189]	Unclear

Out of six potential pathophysiological pathways, five were found to correlate positively with increasing arterial stiffness, forming a theoretical model for the reported strong association between DM2 status and increased PWV. The link between DM2 and AAA is more uncertain, as some mechanism lead to increased risk for aneurysm formation and other to less risk. This could explain why reduced AAA prevalence could not be seen during the early stages of DM2 in paper III. Over time, the negative associations appear to take on a more pronounced role, making patients with DM2 less susceptible to aneurysm development and growth.

Future considerations

The analyses presented in this thesis have all been conducted with statistical methods on data collected from screenings, trials, and national databases. Hence, results do not allow for any inferences regarding what cellular mechanisms, and in certain cases not even what is the cause and what is the effect. The results indicate a continued need for investigation of the associations between glucose processing and blood vessel function and a need for further studies on several relevant themes:

- How aortic stiffness responds to higher glucose concentrations within different age groups.
- More insight into how diabetes protects against aneurysm formation depending on time from diagnosis, aneurysm severity, age and other factors.
- Which indicators that can be used to detect increased risk of aneurysm development.

The results of such analyses could influence future treatment options and lifestyle advice. While current therapy practices are general for all patients with AAA, it could be that a combined DM and AAA diagnosis should lead to a different treatment of AAA in the future. It is hard to imagine that surgical methods could be differentiated, but common advice with regards to nutrition, exercise, and alcohol/smoking might perhaps be modified based on glycemic status? Based on the findings in paper III, one can conclude that AAA screening will be relevant in the future also for men with DM2. Hyperglycemia appears to alleviate some of the dangerous consequences of AAA like rupture or post-operative risks, but it does not remove the risks altogether. If some of the proposed mechanisms connecting DM2 and aneurysm development could be better understood, it could be of substantial importance for mitigating and preventing unwanted dilation of the arteries.

Conclusions

Five papers have been presented, focusing mainly on the associations between deteriorating glucose metabolism, arterial stiffening, and AAA:

Relationships between glycemia and aortic stiffness

- Higher levels of glucose are associated with higher pulse wave velocities, but this is detectable primarily among older individuals (paper I).

Relationships between biomarkers and AAA

- Several selected biomarkers were tested, whereof none could be used to predict aortic diameter (paper II).

Relationships between glycemia and AAA

- No relationships between DM and aortic diameter could be demonstrated in men during the early stages of diabetes (paper III). Patients with DM2 had a lower need for re-intervention after elective EVAR (paper IV), and lower rate of cardiovascular and total mortality after acute AAA surgery (paper V) than patients without diabetes.

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Stiffness and aneurysm of the aorta

Soumia Taimour was born in Morocco and received her Medical Degree at Universite Mohammed V in Rabat. After moving to Sweden, she gained a Swedish Doctor's License in 2004 and has since passed specialist diplomas in Internal Medicine (2011) and Endocrinology and Diabetology (2013) and sees it as a privilege to have served as a physician in Region Skåne health care for more than 20 years in total. She wishes to encourage all professional immigrant women to be proud, work hard to overcome hurdles and attain their goals.

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