

KAIDO PAAPSTEL

Metabolomic profile of arterial
stiffness and early biomarkers of
renal damage in atherosclerosis



DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

248

KAIDO PAAPSTEL

Metabolomic profile of arterial
stiffness and early biomarkers of
renal damage in atherosclerosis



Department of Biochemistry, Institute of Biomedicine and Translational Medicine; Centre of Excellence for Genomics and Translational Medicine; University of Tartu, Tartu, Estonia.
Department of Cardiology, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia.

The dissertation has been accepted for the commencement of the degree of Doctor of Philosophy in Medicine on September 21st 2016 by the Council of the Faculty of Medicine, University of Tartu, Estonia.

Supervisors: Senior Research Fellow Jaak Kals, MD, PhD
Department of Biochemistry, Institute of Biomedicine and Translational Medicine; Centre of Excellence for Genomics and Translational Medicine;
Department of Surgery, Institute of Clinical Medicine; University of Tartu, Tartu, Estonia

Professor Mihkel Zilmer, PhD
Department of Biochemistry, Institute of Biomedicine and Translational Medicine; Centre of Excellence for Genomics and Translational Medicine;
University of Tartu, Tartu, Estonia

Professor Jaan Eha, MD, PhD
Department of Cardiology, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia

Reviewers: Professor Vallo Tillmann, MD, PhD
Department of Paediatrics, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia

Professor Vallo Volke, MD, PhD
Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

Opponent: Professor Bengt C Fellström, MD, PhD
Department of Medical Sciences, Renal Unit
Uppsala University Hospital
Uppsala University, Uppsala, Sweden

Commencement: December 2nd 2016

Publication of this dissertation is granted by the University of Tartu.
This research was supported by the European Union through the European Social Fund.



European Union
European Social Fund



Investing in your future

ISSN 1024–395X
ISBN 978-9949-77-275-9 (print)
ISBN 978-9949-77-276-6 (pdf)

Copyright: Kaido Paapstel

University of Tartu Press
www.tyk.ee

To my beloved ones

CONTENTS

LIST OF ORIGINAL PUBLICATIONS	9
ABBREVIATIONS.....	10
1. INTRODUCTION.....	12
2. REVIEW OF THE LITERATURE.....	14
2.1. Vascular function and cardiovascular disease	14
2.1.1. Arterial stiffness and its determinants	14
2.1.2. Measures of arterial stiffness and central hemodynamics	15
2.1.2.1. Pulse wave analysis.....	15
2.1.2.2. Pulse wave velocity.....	16
2.1.3. Prognostic value of arterial stiffness.....	17
2.1.4. Arginine and asymmetric dimethylarginine	17
2.2. Renal damage and cardiovascular risk	18
2.2.1. Neutrophil gelatinase-associated lipocalin	19
2.2.2. Liver-type fatty acid-binding protein.....	19
2.2.3. Kidney injury molecule-1	19
2.2.4. Cystatin C	20
2.2.5. Fibulin-1	20
2.3. Inflammation and cardiovascular risk	21
2.3.1. Inflammation-related biomarkers	21
2.3.2. Adipokines and plasminogen activator inhibitor-1	23
2.3.3. Oxidized low-density lipoprotein	26
2.4. Metabolic profiling of lipid metabolism in cardiovascular disease...	27
2.4.1. Traditional lipid biomarkers of cardiovascular risk.....	27
2.4.2. Metabolomics and novel lipid biomarkers of cardiovascular risk	28
2.4.2.1. Acylcarnitines	28
2.4.2.2. Phosphatidylcholines	29
2.4.2.3. Lysophosphatidylcholines.....	30
3. AIMS OF THE THESIS	32
4. SUBJECTS AND METHODS.....	33
4.1. Study population.....	33
4.1.1. Patients with symptomatic coronary artery disease and patients with symptomatic peripheral arterial disease	33
4.1.2. Clinically healthy subjects.....	33
4.2. Study design and protocol	33
4.3. Methods	34
4.3.1. Biochemical analysis of blood and urine.....	34
4.3.2. Targeted serum metabolite profiling	34
4.3.3. Peripheral blood pressure measurement	37
4.3.4. Assessment of arterial stiffness and central hemodynamics....	37

4.3.4.1. Pulse wave analysis.....	37
4.3.4.2. Pulse wave velocity.....	37
4.3.5. Measurement of angiographic score.....	38
4.3.6. Statistical analysis.....	38
5. RESULTS	40
5.1. Relationships between serum fibulin-1, arterial stiffness and inflammation in patients with atherosclerosis and in healthy subjects (Paper I).....	40
5.2. Early biomarkers of renal damage in relation to arterial stiffness and inflammation in coronary artery disease patients and in healthy subjects (Paper II).....	43
5.3. Serum short- and medium-chain acylcarnitines independently determine aortic stiffness in coronary artery disease patients (Paper III)	47
5.4. Serum phosphatidylcholines and lysophosphatidylcholines are inversely related to aortic stiffness, endothelial dysfunction and heart rate in patients with atherosclerosis (Papers III and IV).....	52
6. DISCUSSION	59
6.1. Relationships between biomarkers of renal damage, arterial stiffness and inflammation in atherosclerotic patients without moderate to severe chronic kidney disease (Papers I and II).....	59
6.2. Arterial stiffness, hemodynamics and serum acylcarnitines in patients with coronary artery disease (Paper III).....	62
6.3. Arterial stiffness, endothelial dysfunction and resting heart rate in relation to serum phosphatidylcholines and lysophosphatidylcholines in patients with atherosclerosis (Papers III and IV).....	64
6.4. Possible reasons for observed differences between coronary artery disease patients and peripheral arterial disease patients (Papers I and IV)	66
6.5. Limitations.....	67
7. CONCLUSIONS.....	68
8. REFERENCES.....	69
SUMMARY IN ESTONIAN	100
ACKNOWLEDGEMENTS	103
PUBLICATIONS.....	105
CURRICULUM VITAE	157
ELULOOKIRJELDUS.....	159

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by their Roman numerals (I–IV):

- I Paapstel K, Zilmer M, Eha J, Tootsi K, Piir A, Kals J. Association between fibulin-1 and aortic augmentation index in male patients with peripheral arterial disease. *Eur J Vasc Endovasc Surg* 2016; 51(1):76–82.
- II Paapstel K, Zilmer M, Eha J, Tootsi K, Piir A, Kals J. Early biomarkers of renal damage in relation to arterial stiffness and inflammation in male coronary artery disease patients. *Kidney Blood Press Res* 2016; 41(4):488–97.
- III Paapstel K, Kals J, Eha J, Tootsi K, Ottas A, Piir A, Zilmer M. Metabolic profiles of lipid metabolism, arterial stiffness and hemodynamics in male coronary artery disease patients. *IJC Metab Endocr* 2016; 11:13–18.
- IV Paapstel K, Kals J, Eha J, Tootsi K, Ottas A, Piir A, Jakobson M, Lieberg J, Zilmer M. Serum phosphatidylcholines and lysophosphatidylcholines are inversely related to aortic stiffness, endothelial dysfunction and heart rate in male patients with symptomatic atherosclerosis. (submitted for publication)

Author's contribution:

Papers I–IV: Involvement in the study design, collecting clinical data, data analysis, and writing the paper

ABBREVIATIONS

a	acyl
aa	diacyl
ae	acyl-alkyl
ACE	angiotensin-converting enzyme
ADMA	asymmetric dimethylarginine
AIx	augmentation index
AIx@75	augmentation index corrected for a heart rate of 75 beats/minute
AKI	acute kidney injury
AP	augmentation pressure
ARB	angiotensin receptor blocker
Arg	arginine
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CDBP	central diastolic blood pressure
cf-PWV	carotid-femoral pulse wave velocity
CKD	chronic kidney disease
CoA	coenzyme A
CPP	central pulse pressure
CPT	carnitine palmitoyltransferase
Creat	creatinine
cr-PWV	carotid-radial pulse wave velocity
CRP	C-reactive protein
CSBP	central systolic blood pressure
CVD	cardiovascular disease
Cx:y	x denotes the number of carbons in the fatty acid side chains and y denotes the number of double bonds
CysC	cystatin C
DC	decarboxyl
ECG	electrocardiogram
ED	endothelial dysfunction
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
FBLN-1	fibulin-1
HDL	high-density lipoprotein
hsCRP	high sensitivity C-reactive protein
IL-6	interleukin-6
IL-6R	IL-6 receptor
IMT	intima-media thickness
KIM-1	kidney injury molecule-1
L-FABP	liver-type fatty acid-binding protein

LDL	low-density lipoprotein
log	logarithmic
lysoPC a Cx:y	lysophosphatidylcholine
MAP	mean arterial pressure
Met	methionine
Met-SO	methionine sulfoxide
MI	myocardial infarction
MPO	myeloperoxidase
n-6	omega-6 fatty acid
n-9	omega-3 fatty acid
NGAL	neutrophil gelatinase-associated lipocalin
NO	nitric oxide
NOS	nitric oxide synthase
OH	hydroxyl
OxLDL	oxidized low-density lipoprotein
OxS	oxidative stress
P ₁	first systolic peak
P ₂	second systolic peak
PAD	peripheral arterial disease
PAI-1	plasminogen activator inhibitor-1
PC	phosphatidylcholine
PC aa Cx:y	diacyl-phosphatidylcholine
PC ae Cx:y	acyl-alkyl-phosphatidylcholine
PCA	principal component analysis
PDBP	peripheral diastolic blood pressure
PLT	platelet
PP	pulse pressure
PPP	peripheral pulse pressure
PSBP	peripheral systolic blood pressure
PUFA	polyunsaturated fatty acid
PWA	pulse wave analysis
PWV	pulse wave velocity
RCT	randomized controlled trial
SM	sphingomyelin
SM (OH) Cx:y	hydroxysphingomyelin
TNF- α	tumor necrosis factor alpha
WBC	white blood cell

1. INTRODUCTION

Atherosclerosis is a chronic lipid-driven inflammatory disorder and the dominant underlying cause of cardiovascular disease (CVD) (Libby *et al.* 2009; Wong 2014). Early detection of risk factors and manifestations of atherosclerosis is crucial, since CVD accounts for nearly one-third of all deaths worldwide (Wong 2014). Atherosclerotic lesions occur principally in large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain or extremities (Ross 1999). These arteries expand and contract in response to the forces induced by the pulsatile flow, and thus serve both a conduit and a buffering function. Arterial stiffness is a general term for viscoelastic properties (elasticity, distensibility, compliance, etc.) of the arteries and is one of the earliest detectable signs of the structural and functional alterations of the vessel wall (Laurent *et al.* 2006). Changes in arterial stiffness seem to associate with certain hemodynamic, biochemical and inflammatory alterations (Chue *et al.* 2010; Jatoi *et al.* 2007; McEniery *et al.* 2010a; Wilkinson *et al.* 2002; Woodman *et al.* 2005). Detailed insights into these associations may help to better understand the mechanisms of decline in vascular function, potentially leading to CVD.

Reductions in renal function even in the normal or mildly impaired range (estimated glomerular filtration rate (eGFR) 60–130 mL/min/1.73 m²) are independently associated with a significant increase in incident CVD (Eisen *et al.* 2015). Changes in arterial stiffness could at least partially contribute to early functional and structural kidney damage. Since elevated serum creatinine is a relatively late marker of renal impairment (Waikar *et al.* 2012; Wu and Parikh 2008), a number of potential candidate biomarkers for early detection of renal damage have been proposed in recent years (Charlton *et al.* 2014; Neiman *et al.* 2011). Among these are neutrophil gelatinase-associated lipocalin (NGAL), liver-type fatty acid-binding protein (L-FABP), kidney injury molecule-1 (KIM-1), cystatin C (CysC) and fibulin-1 (FBLN-1). Each of these biomarkers have been reported to have a potential utility for early identification of acute kidney injury (AKI) (Basu *et al.* 2014; Fiseha 2015; Ichimura *et al.* 1998, 2004; Mishra *et al.* 2003; Neiman *et al.* 2011; Parr *et al.* 2015). Moreover, several studies have shown that these proteins may also provide a prognostic value for CV morbidity and mortality (Cangemi *et al.* 2011; Carlsson *et al.* 2013, 2014, Lindberg *et al.* 2012, 2014; Matsumori *et al.* 2012). The role of these biomarkers in atherosclerotic patients with an eGFR of more than 60 mL/min/1.73 m² (i.e. without moderate to severe chronic kidney disease (CKD)) is uncertain. However, it can be hypothesized that individuals with elevated levels of early renal damage markers may also have worse vascular function. Thus, in addition to their role in detection of renal impairment, these proteins may also prove useful as sensitive reflectors of the interplay between renal and CV dysfunctions. Yet, it must be acknowledged that some of these markers have also been suggested to directly participate in the development of atherosclerosis, independently of their

involvement in renal damage (Argraves *et al.* 2009; Galis and Khatri 2002; Hemdahl *et al.* 2006; Zhu *et al.* 2015).

Although the lipid metabolism-related classical biomarkers (i.e. total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides) and glucose have been estimated for decades to assess the risk for CVD, recent analytical developments may enable to extend the current metabolic signature for both CVD prognosis and risk stratification.

Metabolomics is an emerging discipline for profiling low molecular weight metabolites (including amino acids, peptides, lipids, carbohydrates, nucleic acids, fatty acids) in health and disease. Assessment of the metabolome could provide insight into the actual metabolic and physiological state of a specific cell, organ, or organism. This information allows to gain a more profound understanding of the pathogenic mechanisms that lead to CVD, among others. Linking metabolomic data to known and validated clinically relevant biochemical and functional biomarkers is essential in unlocking the true potential of this discipline. Thus, metabolomic profiling of arterial stiffness and hemodynamics could help to identify novel CVD-related biomarkers and reveal potential targets for treatment.

In the present thesis, patients with symptomatic coronary artery disease (CAD) and symptomatic peripheral arterial disease (PAD) were compared. CAD and PAD are among the most common clinical manifestations of atherosclerosis and serve as major public health problems (Criqui and Aboyans 2015; Wong 2014). However, although both of these diseases are related to biochemical and functional abnormalities of the CV system (Abdulhannan *et al.* 2012; Hansson 2005; Mattace-Raso *et al.* 2006; Safar 2007a), a number of distinct differences between patients with PAD and CAD should be acknowledged (Grenon *et al.* 2013; Leng *et al.* 1995; Rice and Lumsden 2006; Shammas 2007). We aimed to study the metabolomic profile of arterial stiffness and the role of early biomarkers of renal damage in both of these patient groups as well as in clinically healthy subjects.

2. REVIEW OF THE LITERATURE

2.1. Vascular function and cardiovascular disease

Recent scientific research has shed light on many secrets of the vascular function. The knowledge gained from these studies leaves no doubt that arterial stiffness is an important early manifestation of CVD (Cecelja and Chowienczyk 2012; Laurent *et al.* 2006; Weber *et al.* 2004). Yet, the precise mechanisms responsible for pathophysiological changes in arterial function still need to be elucidated. Unraveling these mechanisms may ultimately yield novel treatment strategies for arterial stiffening, which in turn could help prevent the development of CV complications.

2.1.1. Arterial stiffness and its determinants

Adverse structural and functional changes within the vessel wall reduce the buffering capacity of arteries and lead to increased arterial stiffness, which is a general term for viscoelastic properties (elasticity, distensibility, compliance, etc.) of the arteries (Van Bortel *et al.* 2012; Laurent *et al.* 2006). The elastic properties of conduit vessels vary along the vascular tree; this variation is due to molecular, cellular, and histological differences in the wall structure between more compliant proximal arteries and stiffer distal arteries (Laurent *et al.* 2006; Nichols and O'Rourke 1998). The pressure load of each heartbeat in large conduit vessels is borne mainly by two extracellular matrix proteins: elastin and collagen (Townsend *et al.* 2015a). Because of the anatomic arrangement of the compliant elastin fibers and stiffer collagenous fibers, elastin bears most of the load at low pressures, whereas collagen is engaged at higher pressures (Townsend *et al.* 2015a; Wolinsky and Glagov 1964). Therefore, both proteins serve as important structural components of arterial stiffness.

Besides the passive mechanical effects of elastin and collagen, arterial stiffness is also determined by active functional components, namely nitric oxide (NO) bioavailability and vascular smooth muscle tone (Bellien *et al.* 2010; Isabelle *et al.* 2012; Safar *et al.* 2001; Sehgel *et al.* 2013). NO is released by the endothelial cells, which form the inner lining of blood vessels, and plays a central role in the control of vascular tone (Bellien *et al.* 2010). Abnormalities in the production or actions of NO lead to endothelial dysfunction (ED) as well as to abnormal vascular remodeling and stiffening (McEniery *et al.* 2006; Numaguchi *et al.* 1995; Rudic and Sessa 1999).

A number of CV risk factors affect arterial stiffness via modulation of its structural and/or functional components. Age and blood pressure (BP) are considered to be the major determinants of vascular elasticity (Avolio *et al.* 1983; Cecelja and Chowienczyk 2009; McEniery *et al.* 2010b). While elastin fibers show the tendency of fragmentation, calcification and degradation in aging, collagen concentration in the arterial wall layers (the intima, media and

adventitia) increases over time (Kohn *et al.* 2015; Schlatmann and Becker 1977). In addition, age-associated arterial stiffening is also caused by non-enzymatic glycation of collagen (Kohn *et al.* 2015; Sims *et al.* 1996). Renal function is another important determinant of arterial stiffness both in CKD patients (Chue *et al.* 2010; Townsend 2015b) and in subjects with normal GFR (Schillaci *et al.* 2006). Hypertension influences arterial stiffness and wave reflections (McEniery *et al.* 2010b) mostly via induction of large artery wall thickness and remodeling of resistance vessels (Thom 1997). Diminished arterial elasticity in diabetes, however, may result from insulin resistance (Van Dijk *et al.* 2003) and the consequences of hyperglycaemia, including the formation of advanced glycation end-products (Airaksinen *et al.* 1993; Woodman *et al.* 2005). Although hypercholesterolemia has been positively associated with arterial stiffness (Wilkinson *et al.* 2002), there have also been conflicting findings (Dart *et al.* 2004; Wilkinson and Cockcroft 2007). Other notable determinants of arterial stiffness include smoking (Jatoi *et al.* 2007), obesity (Wildman *et al.* 2003) and poor cardiopulmonary fitness (Boreham *et al.* 2004).

2.1.2. Measures of arterial stiffness and central hemodynamics

2.1.2.1. Pulse wave analysis

The arterial pressure waveform is a composite of the forward pressure wave created by ventricular contraction and a reflected wave originating from vascular branch points or sites of impedance mismatch (Laurent *et al.* 2006). Pulse wave analysis (PWA) is a computerized process that enables to generate the central aortic pressure waveform from the peripheral wave, which is recorded noninvasively by applanation tonometry in the radial or carotid artery (O'Rourke *et al.* 2001). From the aortic waveform central systolic BP (CSBP), central diastolic BP (CDBP), central pulse pressure (CPP) as well as central augmentation pressure (AP) and augmentation index (AIx) can be calculated (Figure 1).

In elastic arteries, the reflected wave returns to the central aorta in diastole and, therefore, enhances diastolic perfusion pressure in the coronary circulation (Mitchell *et al.* 2004). Reduction in the buffering capacity of the large arteries, however, leads to an early return of reflected waves, and thus to increased central aortic pressure (and hence cardiac workload) along with impaired coronary artery perfusion (Mitchell 2008; Safar 2007b). An alternative hypothesis, however, states that the magnitude of the AP is mainly determined by the arterial reservoir, not wave reflection (Davies *et al.* 2010; McEniery *et al.* 2014). Although intriguing, this view has also received criticism (Mynard *et al.* 2012; Segers *et al.* 2012). Whatever its origin, the phenomenon of left ventricular late-systolic loading can be quantified using the AIx – defined as the difference between the second (P_2) and first (P_1) systolic peaks of the central arterial waveform, expressed as a percentage of the CPP (Laurent *et al.* 2006; Wilkinson *et al.* 2000) (Figure 1). Factors that are known to influence AIx include age,

gender, height, heart rate, ejection duration, pulse wave velocity (PWV) and mean arterial pressure (MAP) (Hayward and Kelly 1997; Sharman *et al.* 2009; Wilkinson *et al.* 2000).

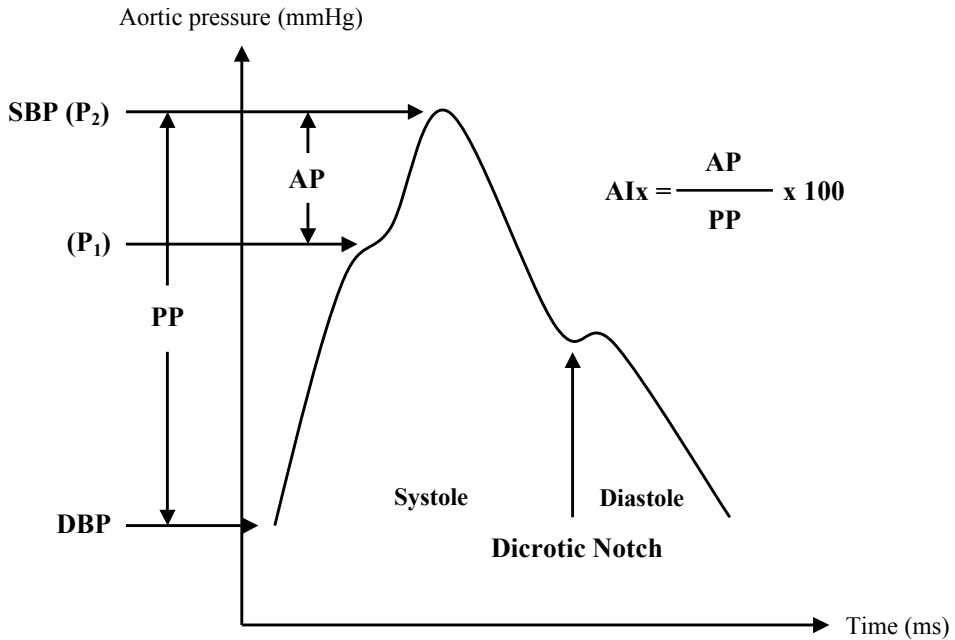


Figure 1. Central aortic waveform and augmentation index (AIx). Abbreviations: AP, augmentation pressure; DBP, diastolic blood pressure; P₁, first systolic peak; P₂, second systolic peak; PP, pulse pressure; SBP, systolic blood pressure. Modified from Hope *et al.* 2002.

2.1.2.2. Pulse wave velocity

The PWV is the rate at which pressure waves travel down the artery. Since the waves propagate faster in stiffer vessels than in elastic ones, PWV is a direct reflector of arterial stiffness (Bramwell and Hill 1922; Laurent *et al.* 2006). Although it can be obtained in different regions of the arterial tree, the carotid-femoral PWV (cf-PWV), a measure of aortic stiffness, is considered the ‘gold standard’ for arterial stiffness assessment (Van Bortel *et al.* 2012; Laurent *et al.* 2006).

Different noninvasive methodologies can be used to determine PWV. These fall under four categories: 1) devices that use a probe or tonometer to record the pulse wave with a transducer, 2) devices using cuffs placed around the limbs or the neck, which record the arrival of the pulse wave oscillometrically, 3) ultrasonography approaches, and 4) magnetic resonance imaging-based approaches (Townsend *et al.* 2015a). Of these, applanation tonometry is probably the most widely used method of cf-PWV measurement. In this method, an arterial

tonometer is used for recording pressure waveforms; pulse transit time is measured from the foot of the carotid waveform to that of the femoral waveform using sequential recordings referenced to the electrocardiogram (ECG); the distance between the two recording sites is measured on body surface and cf-PWV is calculated as the ratio between this distance and the pulse transit time (Laurent *et al.* 2006; Millasseau *et al.* 2005). It is essential to perform accurate measurement of BP at the time of cf-PWV assessment because MAP and age are the two critical determinants of aortic stiffness (Mattace-Raso *et al.* 2010; Townsend *et al.* 2015a).

2.1.3. Prognostic value of arterial stiffness

Numerous studies have demonstrated that aortic stiffness, measured as cf-PWV, independently predicts CV risk and all-cause mortality (Laurent *et al.* 2001; Mitchell *et al.* 2010; Vlachopoulos *et al.* 2010a, 2014; Willum-Hansen *et al.* 2006). Current European guidelines for the management of arterial hypertension state that cf-PWV is a marker of asymptomatic organ damage and can thus help reclassify patients at intermediate risk into a higher or lower CV risk in clinical practice (Ben-Shlomo *et al.* 2014; Mancia *et al.* 2013). The PWV obtained in the carotid-radial segment (cr-PWV) does not seem to have a prognostic value on its own (Laurent *et al.* 2006). However, a prospective study in dialysis population reported that the ratio of cf-PWV and cr-PWV could be a better prognostic predictor of mortality than cf-PWV alone (Covic and Siriopol 2015; Fortier *et al.* 2015).

The AIx has also shown independent associations with CV events (Janner *et al.* 2013; Weber *et al.* 2005; Williams *et al.* 2006) and all-cause mortality (Janner *et al.* 2013; London *et al.* 2001). Notably, a systematic review and meta-analysis reported that the relative risk of total CV events and all-cause mortality for a 10% absolute increase of AIx is 1.318 (95% CI 1.093–1.588) and 1.384 (95% CI 1.192–1.606), respectively (Vlachopoulos *et al.* 2010b). Yet, compared to cf-PWV, the prognostic value of AIx is less well established.

Lastly, CPP and peripheral PP (PPP) are surrogate measures of arterial stiffness, which, together with PP amplification (commonly expressed as PPP/PPP), have shown to be related to CV events (Liu *et al.* 2016; Roman *et al.* 2007) and/or mortality (Benetos *et al.* 2012; Safar *et al.* 2002; Zhao *et al.* 2014) in various populations.

2.1.4. Arginine and asymmetric dimethylarginine

Arginine (Arg) is an amino acid that is involved in various metabolic pathways (Wu and Morris 1998). One of its key functions is to serve as a substrate for a family of enzymes named NO synthases (NOS) (Böger 2007; Förstermann *et al.* 1994). These enzymes catalyze the conversion of Arg to NO and citrulline (Förstermann and Sessa 2012). To date, three different isoforms of NOS have

been identified: endothelial NOS (eNOS), inducible NOS and neuronal NOS (Förstermann *et al.* 1994). The NO produced by eNOS in response to stimulation of mechanoreceptors by the shear stress of the flowing blood is critically important for the homeostasis of vascular tone, for interactions between the arterial wall and circulating blood cells, and for vascular structure (Böger 2007). Thus, Arg availability is essential for preserving normal endothelial function.

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of all three NOS isoforms (Kakimoto and Akazawa 1970; Leiper and Nandi 2011; Vallance *et al.* 1992). It is released following the proteolysis of Arg-methylated proteins and is removed from the body by a combination of renal excretion and metabolism by the dimethylarginine dimethylamino-hydrolase enzymes (Leiper and Nandi 2011; Ogawa *et al.* 1989).

Since ADMA inhibits NO generation, its elevated levels predispose to ED, vasoconstriction and vascular remodeling (Wilcox 2012). Elevated circulating levels of ADMA have been reported from various patient populations (Saitoh *et al.* 2003; Surdacki *et al.* 1999; Yoo and Lee 2001). Furthermore, it independently associates with arterial elasticity (Kals *et al.* 2007) and carotid intima-media thickness (IMT) progression (Furuki *et al.* 2008) as well as with CV risk and mortality (Abedini *et al.* 2010; Krzyzanowska *et al.* 2007; Nijveldt *et al.* 2003; Zoccali *et al.* 2001). In some studies, however, the ADMA/Arg ratio has proven to be a better risk marker than ADMA alone (Anderssohn *et al.* 2012; Notsu *et al.* 2015).

In the present thesis, we investigated possible relationships between ADMA and glycerophospholipids in PAD patients, CAD patients and in clinically healthy subjects (Paper IV).

2.2. Renal damage and cardiovascular risk

The CV and renal dysfunctions are intertwined. The data indicate that reductions in renal function even in the normal or mildly impaired range (eGFR 60–130 mL/min/1.73 m²) are independently associated with a significant increase in incident CVD (Eisen *et al.* 2015). Thus, early functional and structural kidney abnormalities may be associated with alterations in arterial function. A number of biomarkers with the potential for early detection of renal damage have been proposed in recent years, including NGAL, L-FABP, KIM-1, CysC and FBLN-1. Besides their potential role as renal biomarkers, some of these proteins have also been suggested to be associated with the development of atherosclerosis (Argraves *et al.* 2009; Galis and Khatri 2002; Hemdahl *et al.* 2006; Zhu *et al.* 2015).

2.2.1. Neutrophil gelatinase-associated lipocalin

The NGAL is a small 25 kDa protein that was originally identified in neutrophils, but is also expressed in various epithelial cells (e.g. kidney, liver, lungs) in response to different pathologic states (Kjeldsen *et al.* 1993; Singer *et al.* 2013; Xu *et al.* 1994). NGAL synthesis becomes upregulated during AKI (Mishra *et al.* 2003), inflammation (Cowland *et al.* 2003), infection (Björkqvist *et al.* 2004), and neoplastic development (Bolognani *et al.* 2010), among others. It is also an essential component of the antimicrobial innate immune system, since it modulates neutrophil functions and sequesters iron-loaded bacterial siderophores (Nasioudis and Witkin 2015). Interestingly, NGAL synthesis is up-regulated in atherosclerosis (Hemdahl *et al.* 2006) and, via formation of a complex with matrix metalloproteinase-9, might participate in plaque rupture (Galis and Khatri 2002; Lindberg *et al.* 2014). Moreover, plasma NGAL correlates with CAD severity (Katagiri *et al.* 2015) and has a prognostic value in patients with acute myocardial infarction (MI) (Lindberg *et al.* 2012) and heart failure (Maisel *et al.* 2011) as well as in the general population (Lindberg *et al.* 2014).

2.2.2. Liver-type fatty acid-binding protein

The FABPs are a large family of small (~15 kDa) cytosolic proteins that participate in the fatty acid uptake, intracellular transport and metabolism (Murphy *et al.* 1996; Ockner *et al.* 1972; Thumser *et al.* 2014). The L-FABP is one of the members of this family and is expressed not only in the liver but also in the intestine, pancreas, stomach, lungs and kidneys (Smathers and Petersen 2011). It has high affinity and capacity to bind long-chain fatty acid oxidation products and is therefore probably an important endogenous antioxidant (Matsui *et al.* 2011; Wang *et al.* 2005). Plasma and/or urinary levels of L-FABP appear to be of a diagnostic and prognostic value for both acute and chronic kidney injury (Fiseha 2015; Mou *et al.* 2012; Parr *et al.* 2015; Susantitaphong *et al.* 2013). Moreover, a study in subjects with type 2 diabetes and CKD suggested that simultaneous measurement of urinary L-FABP and albumin-to-creatinine ratio may be useful to assess cardiac damage in these patients (Maeda *et al.* 2014). In cases of acute coronary syndrome, elevated L-FABP levels can help identify individuals at high risk for future CV events (Matsumori *et al.* 2012).

2.2.3. Kidney injury molecule-1

The KIM-1 is a type 1 transmembrane protein that is not detectable in normal kidney tissue but is highly expressed in dedifferentiated proximal tubule epithelial cells after ischemic (Ichimura *et al.* 1998) or toxic (Ichimura *et al.* 2004) injury. Interestingly, it facilitates clearance of the apoptotic debris from the tubular lumen, and is therefore unique in being the first non-myeloid phosphatidylserine receptor that transforms epithelial cells into semi-professional phagocytes

(Bonventre 2009; Ichimura *et al.* 2008). A study in mice demonstrated that by facilitating the phagocytic process, KIM-1 might have a protective anti-inflammatory role in the early stages of AKI (Yang *et al.* 2015). Another work in a rat MI model showed that KIM-1 gene expression was dramatically up-regulated at 1 week in a post-MI kidney (Lekawanvijit *et al.* 2012). Most importantly, KIM-1 has demonstrated a diagnostic value as an early biomarker of AKI in different clinical settings (Han *et al.* 2009; Medić *et al.* 2015; Yang *et al.* 2016) and was independently associated with CV mortality and incidence of heart failure in a community-based cohort of elderly men (Carlsson *et al.* 2013, 2014).

2.2.4. Cystatin C

The CysC is a 13 kDa endogenous cysteine proteinase inhibitor that is freely filtrated through the glomeruli and has been reported superior to creatinine in identifying renal dysfunction at eGFR levels above 60 mL/min/1.73 m² (Dhar-nidharka *et al.* 2002; Grubb and Löfberg 1982; Luo *et al.* 2015). Moreover, serum CysC helps to improve diagnostic precision for AKI for both adults and children (Basu *et al.* 2014; Peco-Antić *et al.* 2013). A meta-analysis of 38,854 participants showed that elevated serum CysC levels independently associate with excessive CV and all-cause mortality risk in the general populations with age over 40 years (Luo *et al.* 2015). Similarly, a study in middle-aged individuals without a history of CVD reported CysC to be a better risk marker for CV prognosis than creatinine-based GFR (Svensson-Färbom *et al.* 2014). The association between serum CysC and CV events is not entirely explained by the renal dysfunction and might be partially mediated by inflammation (Koenig *et al.* 2005; Salgado *et al.* 2013). Indeed, high CysC concentrations have been previously related to both inflammation (Knight *et al.* 2004; Leung-Tack *et al.* 1990) and atherosclerosis (Zhu *et al.* 2015). However, contrary results have also been observed (Albert *et al.* 2001; Grubb *et al.* 2011).

2.2.5. Fibulin-1

The FBLN-1 is an extracellular matrix glycoprotein that binds fibronectin, elastin, and proteoglycans and is one of the few extracellular matrix proteins normally present at high concentrations in blood (Argraves *et al.* 1989; Cangemi *et al.* 2011). It is notably expressed in the dermis, lung, heart valves and in the blood vessel wall, and seems to be crucial in embryonic development (Miosge *et al.* 1996; Roark *et al.* 1995). Mice lacking FBLN-1 gene expression die perinatally due to a combination of blood loss and renal and respiratory impairments (Kostka *et al.* 2001). Interestingly, plasma profiling has revealed that FBLN-1 could serve as a potential indicator to monitor kidney malfunction or kidney damage (Neiman *et al.* 2011). Since prominent deposition of FBLN-1 has been found within atherosclerotic lesions and clots, the protein may also play a role in

processes leading to the progression and thrombotic complications of atherosclerosis (Argraves *et al.* 2009). Furthermore, FBLN-1 correlates with aortic stiffness and predicts mortality in patients with type 2 diabetes (Cangemi *et al.* 2011; Laugesen *et al.* 2013). In CAD patients without diabetes, a relationship between aortic Aix and plasma FBLN-1 was recently found (Hansen and Rasmussen 2015).

In the current thesis, we aimed to measure the serum/urinary levels of the above-mentioned early biomarkers of renal damage and to evaluate their association with arterial stiffness and inflammation in patients with CAD and in clinically healthy subjects (Papers I and II).

2.3. Inflammation and cardiovascular risk

Inflammation is considered critical for the initiation and progression of atherosclerosis. Moreover, a significant decrease in aortic stiffness following a reduction in inflammation has been reported (Mäki-Petäjä *et al.* 2006). However, whether targeted inhibition of inflammation reduces the risk of CV events still remains unproven. Two ongoing randomized controlled trials (RCTs) address this uncertainty and will hopefully provide good evidence for supporting or rejecting the inflammatory hypothesis of atherothrombosis (Ridker 2009a; Ridker *et al.* 2011).

2.3.1. Inflammation-related biomarkers

Interleukin-6 (IL-6) and C-reactive protein (CRP). The IL-6 is a proinflammatory cytokine that was originally cloned in 1986 (Hirano *et al.* 1986). It exerts its pleiotropic biological actions via a complex consisting of a specific IL-6 receptor (IL-6R) and a signal transducing subunit (glycoprotein 130) (Peters *et al.* 1996). The soluble form of IL-6R is also able to bind with this cytokine, after which the complex can attach to glycoprotein 130. This process is called trans-signaling and it leads to activation of cells that lack membrane-bound IL-6R (Peters *et al.* 1996; Rose-John 2012).

The IL-6 plays critical roles in the immune response and hematopoiesis (Hirano *et al.* 1986, 1990). Moreover, it is a major regulator of acute phase protein synthesis in human hepatocytes, since it stimulates C-reactive protein, serum amyloid A, fibrinogen, haptoglobin and hepcidin production and inhibits the synthesis of transferrin, fibronectin and albumin (Castell *et al.* 1989), among others. Thus, IL-6 is an upstream regulator that plays a central role in propagating the downstream inflammatory response and might therefore make a causal contribution to the development of atherosclerosis (Hartman and Frishman 2014). Large amounts of IL-6 have been found in arterial atherosclerotic lesions (Rus *et al.* 1996; Seino *et al.* 1994) and its circulating concentrations link to both ED (Esteve *et al.* 2007) and arterial stiffness (Mahmud and Feely 2005).

Furthermore, an increased CV risk has been reported in apparently healthy men (Ridker *et al.* 2000a) and women (Ridker *et al.* 2000b) with elevated levels of IL-6 and high-sensitivity CRP (hsCRP). Mendelian randomization studies have suggested that on the basis of genetic evidence, IL-6 seems to have a causal role in development of CVD, whereas CRP does not (Casas *et al.* 2006; Hingorani and Casas 2012; Kivimäki *et al.* 2007; Wensley *et al.* 2011). Therefore, IL-6, rather than CRP, has emerged as a potential therapeutic target in atherothrombotic disease, although moving even further upstream to the interleukin-1 signaling pathway might be needed for efficient immune modulation and atherothrombotic protection (Ridker 2016).

As noted above, CRP is produced by hepatocytes largely under regulatory control of inflammatory cytokines including IL-6 and tumor necrosis factor- α (TNF- α) (Ridker 2009b). It is a homopentameric protein with Ca-binding specificity for phosphocholine and was first discovered in 1930 (Tillett and Francis 1930; Volanakis 2001). The CRP synthesis is rapidly upregulated in response to inflammation and the protein participates in complement activation as well as in innate immune function (Du Clos 2000; Ridker 2016). Interestingly, CRP has been found to induce expression of adhesion molecules (Pasceri *et al.* 2000) and plasminogen activator inhibitor-1 (PAI-1) (Devaraj *et al.* 2003) by human endothelial cells. Furthermore, it also seems to directly decrease eNOS expression/bioactivity (Venugopal *et al.* 2002) and prostacyclin release (Venugopal *et al.* 2003) from these cells. Although these findings suggest that CRP might play a causal role in atherogenesis, the above-mentioned more recent Mendelian randomization studies do not support this view (Kivimäki *et al.* 2007; Wensley *et al.* 2011). Moreover, it has been demonstrated that intact authentic human CRP itself does not have any detectable proinflammatory effects in healthy adults (Lane *et al.* 2014). Nevertheless, hsCRP can still be a clinically useful tool to assess risk in patients with an unusual or moderate CVD risk profile (Perk *et al.* 2012) and might help to determine whether to initiate 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) therapy for primary prevention in selected patients (Stone *et al.* 2014). Like IL-6, hsCRP levels predict future vascular risk in apparently healthy populations (Ridker *et al.* 2000a; Ridker *et al.* 2000b) and associate with both ED (De Haro *et al.* 2008) and arterial stiffness (Kampus *et al.* 2006; Mahmud and Feely 2005). Also, since there is no clinically approved assay for IL-6, and because its measurement is more complicated than that of hsCRP (e.g. circadian variation, shorter half-life, post-prandial effects) (Ridker 2016), the latter remains the biomarker of choice to assess low-grade inflammation in clinical practice.

Myeloperoxidase (MPO) and ferritin. Several other proteins, besides IL-6 and hsCRP, have shown value as inflammatory biomarkers in CVD (Ikonomidis *et al.* 2012; Koenig *et al.* 2005). MPO, a member of the heme peroxidase superfamily, is secreted primarily on polymorphonuclear neutrophil activation and degranulation and participates in both innate and acquired immunity (Lehrer *et al.* 1969; Odobasic *et al.* 2013, 2016). The protein was first isolated in 1941 (Agner 1941) and has a principal role in promotion of oxidative stress (OxS) at

sites of inflammation (Nicholls and Hazen 2005; Zhang *et al.* 2002). It is highly expressed in atherosclerotic lesions (Daugherty *et al.* 1994) and has been shown to initiate lipid peroxidation (Zhang *et al.* 2002), to promote ED (Eiserich *et al.* 2002) and to contribute to plaque rupture via matrix metalloproteinase activation in the artery wall (Fu *et al.* 2001). Increased circulating levels of this protein have been reported in patients with CAD, and particularly in those experiencing acute coronary syndrome (Ndrepepa *et al.* 2008). Furthermore, MPO does not only independently predict CV mortality in both of these populations (Baldus *et al.* 2003; Heslop *et al.* 2010; Mocatta *et al.* 2007) but also helps to identify healthy individuals with higher future risk of CAD (Meuwese *et al.* 2007). Although previous data have suggested a protective effect of MPO deficiency against MI and atheroma (Kutter *et al.* 2000), convincing evidence of a causal relation between MPO and CVD is still lacking.

The participation of transition metal ions such as iron in the formation of different reactive species has also long been recognized (Halliwell and Gutteridge 1984). Ferritin is the main iron storage compound, and hence a biomarker of iron status, in the body and is present mainly in the reticuloendothelial cells of the liver, spleen, and bone marrow (Walters *et al.* 1973). It is an acute phase protein that becomes highly expressed in conditions marked by uncontrolled cellular proliferation, excessive production of toxic oxygen reactive species and inflammation (Konijn and Hershko 1977; Sung *et al.* 2012). Numerous studies have linked high serum ferritin levels to morbidity and mortality in different clinical settings (Abril-Ulloa *et al.* 2014; Kalantar-Zadeh *et al.* 2001; Maiwall *et al.* 2014). It is associated with higher coronary plaque volume (Battes *et al.* 2014) and MI risk (Salonen *et al.* 1992) for patients with CAD and with mortality (DePalma *et al.* 2010) for patients with PAD, whereas in the general population it has been related to both the presence of coronary artery calcium (Sung *et al.* 2012) and early death (Ellervik *et al.* 2014). However, the mechanisms behind the associations between serum ferritin and different low-grade inflammatory diseases are still in dispute (Kell and Pretorius 2014).

In the present thesis, the serum levels of IL-6 (Papers II–IV), hsCRP (Papers I–IV), MPO (Papers II and III) and ferritin (Paper II) were determined and their possible relationship with early biomarkers of renal damage and/or low molecular weight metabolites was examined.

2.3.2. Adipokines and plasminogen activator inhibitor-1

The role of adipose tissue in low-grade inflammation and metabolism is increasingly acknowledged. Since the discovery of leptin (Zhang *et al.* 1994), hundreds of other bioactive molecules, generally named adipokines, have been shown to be secreted by this tissue (Fasshauer and Blüher 2015). The majority of adipokines exert pro-inflammatory effects on the CV system, whereas a small number of anti-inflammatory adipokines have been recognized as CV protectors (Ohashi *et al.* 2014).

Adiponectin is a major anti-inflammatory adipocyte-secreted protein that acts via two receptor isoforms, adiponectin receptor 1 and 2 (Scherer *et al.* 1995; Yamauchi *et al.* 2003). The protein itself has three isoforms (low-, medium- and high molecular weight) with distinct biological effects (Hattori *et al.* 2008; Kobayashi *et al.* 2004) and the percentage of each isoform per total adiponectin can vary in different populations (Aso *et al.* 2006; Rizza *et al.* 2010). While plasma levels of most proteins produced by adipose tissue tend to elevate along with an increase in total body fat mass, adiponectin levels are paradoxically reduced in obesity (Arita *et al.* 1999). There is also a clear gender difference in circulating adiponectin concentrations (Böttner *et al.* 2004), which can be at least partially explained by the inhibition of its secretion from adipocytes by testosterone (Xu *et al.* 2005).

Adiponectin acts as an endogenous insulin sensitizer by stimulating adenosine monophosphate-activated protein kinase (Yamauchi *et al.* 2002) and is inversely associated with type 2 diabetes (Koenig *et al.* 2006; Lindberg *et al.* 2015). Also, it exerts multiple beneficial effects on the CV system through direct and indirect actions on both cardiac and vascular cells (Caselli *et al.* 2014; Ghantous *et al.* 2015). Adiponectin regulates vascular homeostasis via adenosine monophosphate-activated protein kinase-eNOS and cyclooxygenase-2/prostacyclin regulatory pathways within endothelial cells and is able to confer an anti-inflammatory phenotype in macrophages (Ouchi *et al.* 2012). Low circulating levels of adiponectin have been reported to be associated with impaired endothelium-dependent vasorelaxation (Okui *et al.* 2008; Ouchi *et al.* 2003), arterial stiffness (Youn *et al.* 2013), left ventricular hypertrophy (Pääkkö *et al.* 2010) and hypertension (Imatoh *et al.* 2008). Interestingly, however, a meta-analysis with 23,717 participants showed that, in the general population, serum adiponectin levels were positively related to the risk of ischemic stroke and did not associate with an increased or decreased risk of CVD (Hao *et al.* 2013). The authors speculated that differences in the levels of adiponectin isoforms with variable pathophysiological roles may partially underlie those somewhat unexpected results (Hao *et al.* 2013).

Leptin, resistin and PAI-1. The biological effects of leptin, resistin and PAI-1 are generally considered to be pro-inflammatory under pathophysiological conditions. Leptin is produced primarily in the adipocytes and acts through its receptor (Tartaglia *et al.* 1995) which is present in a wide range of tissues in several alternatively spliced forms (Chen *et al.* 1999; Margetic *et al.* 2002). This adipokine modulates the CV, immune, nervous, and reproductive systems (Abel and Sweeney 2012; Lord *et al.* 1998; Margetic *et al.* 2002), but its key task is the regulation of appetite (Brunner *et al.* 1997) and energy homeostasis (Tuominen *et al.* 1997). In obesity, despite elevated circulating leptin concentrations, a dysregulation of energy balance is observed, suggesting that obese people become resistant to this adipokine (Bjørnbæk *et al.* 1999; Freitas Lima *et al.* 2015). Interestingly, however, the resistance appears to be selective, since insulin desensitizing and pro-inflammatory effects of leptin are maintained (Freitas Lima *et al.* 2015; Mark 2013; Mark *et al.* 2002). Indeed,

increased circulating leptin levels have been associated with insulin resistance (Fischer *et al.* 2002), hypertension (Correia *et al.* 2001), arterial stiffness (Windham *et al.* 2010), myocardial wall thickness (Paolisso *et al.* 1999) and atherosclerosis (Reilly *et al.* 2004; Schäfer *et al.* 2004), among others. Therefore, leptin can be regarded as a biomarker linking obesity and insulin resistance with various CV pathologies.

In 2001, another pro-inflammatory adipocyte-derived hormone, resistin, was discovered and linked with obesity and diabetes in mice (Steppan *et al.* 2001a). However, human resistin is primarily expressed in and secreted from monocytes (Lee *et al.* 2014; Patel *et al.* 2003) and is sometimes referred to as a protein “found in the inflammatory zone” (Fantuzzi 2005). It is a 12.5 kDa cysteine rich peptide (Steppan *et al.* 2001b) whose receptor in humans was unknown until recently, when adenylyl cyclase-associated protein 1 was shown to mediate the pro-inflammatory effects of resistin *in vitro* and *in vivo* (Lee *et al.* 2014). Interestingly, resistin is able to strongly up-regulate IL-6 and TNF- α expression and enhance its own activity by a positive feedback (Bokarewa *et al.* 2005). Its independent association with heart failure incidence and CV risk (Frankel *et al.* 2009; Menzaghi *et al.* 2013; Muse *et al.* 2015) could be explained by its pro-inflammatory and pro-atherogenic effects (Cho *et al.* 2010; Reilly *et al.* 2005). Moreover, resistin has been reported to associate with arterial stiffness (Windham *et al.* 2010) and ED (Solini *et al.* 2012; Verma *et al.* 2003). However, its role in insulin resistance and obesity still remains uncertain in humans (Huang and Yang 2015; McTernan *et al.* 2002; Utschneider *et al.* 2005).

The PAI-1 is a ~50 kDa glycoprotein that belongs to a class of serine protease inhibitors and acts as the primary physiological inhibitor of both the urokinase- and tissue-type plasminogen activators (Van Mourik *et al.* 1984). The protein is expressed in vascular endothelial cells, vascular smooth muscle cells, adipocytes, hepatocytes, platelets (PLTs), monocytes and macrophages, among others (Ha *et al.* 2009). Elevations in plasma PAI-1 levels can be observed in obesity (Landin *et al.* 1990; Solá *et al.* 2008) and may partially explain the increased risk of atherothrombotic events in overweight individuals (Wolk *et al.* 2003). Moreover, an autocrine role for adipocyte PAI-1 in promotion of adipocyte differentiation and lipid accumulation has been suggested (Correia and Haynes 2006; Crandall *et al.* 2006). In addition to its prothrombotic effects (Eitzman *et al.* 2000), PAI-1 also seems to be associated with tissue fibrosis (Ghosh and Vaughan 2012) and atherosclerosis (Schneiderman *et al.* 1992), especially in patients with type 2 diabetes (Pandolfi *et al.* 2001; Sobel *et al.* 1998). Its production by vascular endothelial cells is induced by a number of stimuli including interleukin-1 (Nachman *et al.* 1986) and TNF- α (Van Hinsbergh *et al.* 1988). However, while PAI-1 seems to augment proliferation and to inhibit apoptosis of vascular smooth muscle cells (Chen *et al.* 2006; Rossignol *et al.* 2006), it has also been reported to limit plaque growth and to prevent abnormal matrix remodeling (Luttun *et al.* 2002). Therefore, the true role of this adipokine in vascular remodeling remains controversial (Fay *et al.* 2007; Konstantinides *et*

al. 2002). In clinical studies, elevated circulating PAI-1 levels have been associated with both higher risk of CV events (Smith *et al.* 2005; Takazoe *et al.* 2001) and mortality (Akkus *et al.* 2009). Yet, negative results have also been reported (Pineda *et al.* 2009; Wang *et al.* 2006).

In the current thesis, serum adiponectin (Papers II and III), resistin (Papers I, II and IV) and PAI-1 (Paper I) levels were measured and their potential relationship with early biomarkers of renal damage and/or low molecular weight metabolites was assessed.

2.3.3. Oxidized low-density lipoprotein

An imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage, is termed ‘OxS’ (Sies 1997). Oxidative damage to nucleic acid bases, lipids, and proteins can, in turn, compromise cell health and viability (Dalle-Donne *et al.* 2006). It is therefore not surprising that elevated OxS has been suggested to be involved in the pathogenesis of numerous diseases (Alfadda and Sallam 2012). However, due to various shortcomings with the biomarkers and methods available to assess the OxS status, its pathophysiological significance is often difficult to prove.

Oxidized low-density lipoprotein (OxLDL) has been studied for more than three decades (Henriksen *et al.* 1981; Quinn *et al.* 1987; Steinbrecher *et al.* 1984) and can be defined as follows: a particle derived from circulating LDL that may have peroxides or their degradation products (generated within the LDL molecule or elsewhere in the body) associated with the particle (Parthasarathy *et al.* 2010). The oxidation of LDL is a complex process during which both the protein and the lipids undergo oxidative changes and form complex products (Parthasarathy *et al.* 2010). A major role of OxLDL in promoting atherogenesis through foam cell formation and inflammatory responses is well-documented (Jones *et al.* 2000; Quinn *et al.* 1987; Van Tits *et al.* 2011; Ylä-Herttuala *et al.* 1989). Higher circulating levels of this biomarker have been reported in various populations (Holvoet *et al.* 1998; Weinbrenner *et al.* 2006; Zagura *et al.* 2012). Moreover, elevated OxLDL levels seem to associate with hypertension (Frostedgård *et al.* 2003), arterial stiffness (Zagura *et al.* 2012), carotid artery IMT (Kampus *et al.* 2007), early cardiac damage (Rietzschel *et al.* 2008), high waist circumference (Weinbrenner *et al.* 2006), type 2 diabetes (Njajou *et al.* 2009) and higher risk of future CV events (Meisinger *et al.* 2005; Shimada *et al.* 2004).

In the current thesis, oxLDL levels were measured and their potential relationship with serum FBN-1 (Paper I) and acylcarnitines (Paper III) in PAD patients, CAD patients and in clinically healthy subjects was evaluated.

2.4. Metabolic profiling of lipid metabolism in cardiovascular disease

Alterations in lipid metabolism by itself and via interaction with a variety of other CV risk factors may promote the development of atherosclerotic disease. The levels of traditional lipid metabolism-related biomarkers (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) have been used for decades in clinical practice to assess the risk for CVD. However, recent analytical advancements (e.g. metabolomics) may enable to further extend the current lipidomic signature for both CVD prognosis and risk stratification.

2.4.1. Traditional lipid biomarkers of cardiovascular risk

Cholesterol is an essential structural component of cellular membranes and lipid rafts (Rietveld and Simons 1998), influencing phospholipid bilayer fluidity and permeability (Cooper 1978). It is a precursor molecule for the synthesis of steroid hormones and bile salts and its biosynthesis is also related to the synthesis of vitamin D (Hanukoglu 1992). The blood levels of cholesterol are largely determined by its endogenous synthesis in the liver and, to a lesser extent, by dietary intake (Griffin and Lichtenstein 2013).

Cholesterol is transported through the circulation by particles called blood lipoproteins. LDL particles are the major cholesterol carriers, and are responsible for transporting cholesterol to peripheral tissues. HDL particles, on the contrary, remove excess cholesterol from cells and from some other types of blood lipoproteins and deliver it back to the liver. Multiple RCTs have reported that reducing total cholesterol and LDL cholesterol can prevent CVD (Fulcher *et al.* 2015; Pedersen *et al.* 1994; Reiner *et al.* 2011). Although statin therapy has been credited with various beneficial effects (Wang *et al.* 2008), a large-scale RCT published in 2015 provided the first evidence that LDL cholesterol lowering *per se* can explain the effect of statins on CV outcomes (Cannon *et al.* 2015). In addition, hypercholesterolemia has been positively associated with arterial stiffness (Wilkinson *et al.* 2002; Wilkinson and Cockcroft 2007), although conflicting findings have also been reported (Cecelja and Chowienzyk 2009; Dart *et al.* 2004; Wilkinson and Cockcroft 2007).

The role of HDL cholesterol in CVD is controversial. Observational data suggest that its concentration in blood is inversely associated with CV risk (Di Angelantonio *et al.* 2009; Castelli *et al.* 1986). Mendelian randomization studies (Voight *et al.* 2012) and interventional studies (Barter *et al.* 2007; Boden *et al.* 2011), however, have not shown a causal link between elevated HDL cholesterol and CV protection. Nonetheless, improving HDL functionality, rather than raising HDL cholesterol levels alone, might still prove to be an efficient way to improve CV outcomes in the future (Fazio and Linton 2010; Rohatgi *et al.* 2014).

High total triglyceride levels have long been recognized as a risk factor for CVD (Albrink and Man 1959; Brown *et al.* 1965). A triglyceride molecule consists of three fatty acids bound to a glycerol backbone and serves as an important storage of energy in the body. Recent genetic studies suggest that elevated concentrations of triglyceride-rich lipoproteins or their remnants are causally associated with CVD and all-cause mortality (Do *et al.* 2013; Jørgensen *et al.* 2013; Thomsen *et al.* 2014; Varbo *et al.* 2013). However, it still remains uncertain if lowering triglycerides reduces CVD in hypertriglyceridemic patients.

2.4.2. Metabolomics and novel lipid biomarkers of cardiovascular risk

Systems biology in conjunction with omics techniques (genomics, transcriptomics, proteomics and metabolomics) provide a holistic view of the molecular processes, ranging from a single cell to the whole organism. Metabolomics is focused on chemical processes involving low molecular weight (<1500 Da) metabolites (including amino acids, peptides, lipids, carbohydrates, nucleic acids, fatty acids) which reflect changes in the genome, transcriptome and proteome. Therefore, metabolomics represents the endpoint of the omics cascade and is closest to the actual phenotype. Two distinct approaches, targeted and untargeted metabolomics, can be followed to analyze a set of metabolites in biofluids or tissues. The aim of the targeted approach is to quantify only a pre-selected set of known metabolites based on internal or external reference compounds. Untargeted metabolomics, on the other hand, refers to an analysis of all measurable metabolites in a biological sample. There are advantages and disadvantages to both strategies (Patti *et al.* 2012), and the choice of approach usually depends on the objectives of the experiment.

As might be expected, metabolomic profiling of CVD has become a vibrant field of research. Altered lipid metabolism in atherosclerosis, in particular, has been the focus of a number of recent studies (Meikle *et al.* 2011; Rizza *et al.* 2014; Shah *et al.* 2012; Stegemann *et al.* 2014). The field of lipidomics allows to survey a wide spectrum of lipid species in body fluids/tissues and provides new insights into the pathogenetic mechanisms of atherogenesis. Among these species, acylcarnitines, phosphatidylcholines (PCs) and lysophosphatidylcholines (lysoPCs) have received attention as potential novel independent lipid risk markers of CVD (Ganna *et al.* 2014; Shah *et al.* 2012; Sigruener *et al.* 2014).

2.4.2.1. Acylcarnitines

Conjugation to carnitine is required in order to transport activated long-chain fatty acids across the inner mitochondrial membrane. These carnitine esters are known as acylcarnitines. In the mitochondrial matrix, acylcarnitine reconjugates with a coenzyme-A (CoA) molecule after which reformed acyl-CoA undergoes β -oxidation to produce energy. However, when fatty acid release by adipose

tissue triglycerides exceeds the rate of β -oxidation, or when the oxidative metabolism of fatty acids in mitochondria is impaired (e.g. different inborn errors of mitochondrial β -oxidation (Vianey-Liaud *et al.* 1987; Wanders *et al.* 1999)), the concentrations of different circulating acylcarnitines will be increased. An elevation in the levels of intramyocardiocellular long-chain acylcarnitines may also be induced by hypoxia (McHowat *et al.* 1993).

Metabolomic profiles, composed of dicarboxylacylcarnitines, medium-chain acylcarnitines, and fatty acids, have been previously found to be independently predictive of future CV events and may improve risk discrimination beyond the degree possible using readily available clinical characteristics (Shah *et al.* 2012). A small study in elderly patients with a high rate of previous history of CAD also reported an independent association between medium- and long-chain acylcarnitines and the subsequent occurrence of CV events (Rizza *et al.* 2014). Furthermore, higher circulating levels of long-chain acylcarnitines are independently predictive of the functional status and mortality in patients with chronic systolic heart failure (Ahmad *et al.* 2016). Importantly, these abnormalities seem to be modifiable with left ventricular assist device support in end-stage heart failure patients (Ahmad *et al.* 2016).

Taken together, these findings clearly indicate that medium- and long-chain acylcarnitines are associated with CV risk. However, whether the accumulation of these lipid species appears due to a metabolic shift toward myocardial fatty acid oxidation inhibition along with greater utilization of glucose (Neely and Morgan 1974), insulin resistance (Schooneman *et al.* 2013), defects in the 'carnitine shuttle' leading to mitochondrial dysfunction (Ahmad *et al.* 2016), or other unknown mechanisms remains to be elucidated.

In the current thesis, the serum levels of acylcarnitines were determined and their possible relationship with arterial stiffness, hemodynamics and inflammation in patients with CAD and in clinically healthy subjects was evaluated (Paper III).

2.4.2.2. Phosphatidylcholines

The PCs are a class of glycerophospholipids which serve as structural components in cellular membranes, blood lipoproteins, natural surfactants and bile, among others. A PC molecule can have numerous different combinations of fatty acids of varying lengths and saturation bound to the sn-1 (mostly saturated and monounsaturated fatty acids) and sn-2 (mostly polyunsaturated fatty acids) positions of the glycerol backbone, although those of 16-, 18- or 20-carbon chain length are the most common. For instance, a combination of myristic acid (C14:0) and linoleic acid (C18:2) at sn-1 and sn-2 positions, respectively, or palmitoleic acid (C16:1) at both sn-1 and sn-2 positions, can compose a PC aa C32:2 molecule. Some fatty acids attached to the glycerol moiety (e.g. arachidonic acid, docosahexaenoic acid) serve as essential precursors to lipid-derived signaling molecules (Wymann and Schneider 2008). Moreover, PCs are also reservoirs

and transporters of phosphate, glycerol and choline. The functional properties and oxidative susceptibility of individual PC molecules are largely determined by the diverse composition and distribution of saturated and unsaturated fatty acids on the glycerol backbone (Philippova *et al.* 2014). Alterations to PC molecular species composition can, in turn, result from an underlying (patho)physiological state (Delaš *et al.* 2008; Engelmann *et al.* 1992; McLeod and Sevanian 1997).

A study in individuals hospitalized for coronary angiography showed that PC species containing long chain saturated and monounsaturated omega-9 (n-9) fatty acids were positively associated with mortality while long-chain polyunsaturated fatty acids appeared to be associated with a protective effect in these patients (Sigruener *et al.* 2014). The authors considered it highly likely that the protective lipid species contain arachidonic acid (20:4 n-6) (Sigruener *et al.* 2014); this view is supported by another study showing reduced CAD risk in subjects with an increased 20:4 (n-6) to 20:3 (n-6) ratio (i.e. increased delta-5 desaturase activity) (Lu *et al.* 2012).

In 2011, a positive relationship between gut-flora-dependent metabolism of dietary PC and atherosclerosis was discovered in mice (Wang *et al.* 2011). Gut bacteria metabolize the choline group of PC to trimethylamine which is further converted into pro-atherogenic trimethylamine-N-oxide in the liver. Subsequent large-scale human studies showed independent associations between elevated circulating trimethylamine-N-oxide and an increased risk of incident major adverse CV events (Tang *et al.* 2013; Wang *et al.* 2014) and thus further indicated a detrimental effect of trimethylamine-N-oxide on the CV system.

In the present thesis, the serum concentrations of PCs were measured and their potential relationship with arterial stiffness, hemodynamics, ED and inflammation in CAD patients, PAD patients and clinically healthy subjects was examined (Papers III and IV)

2.4.2.3. Lysophosphatidylcholines

If PC molecule becomes partially hydrolyzed by phospholipase A2 or phospholipase A1, one of the two fatty acids bound to the glycerol backbone is removed, and lysoPC is generated. The production of lysoPC can also result from lecithin-cholesterol acyltransferase activity (Subbaiah *et al.* 1980) or hepatic secretion (Sekas *et al.* 1985). These lipid species are major components of oxLDL and whereas some of these species seem to possess pro-atherogenic properties, others may have anti-atherogenic qualities (Aiyar *et al.* 2007; Hara *et al.* 1997; Schmitz and Ruebsaamen 2010). Like PCs, lysoPCs also serve as reservoirs and transporters of phosphate, glycerol and choline. Moreover, both lipid classes participate in cell signaling through multiple G protein-coupled receptors that regulate a wide range of cellular functions (Hara *et al.* 1997; Matsumoto *et al.* 2006; Schmitz and Ruebsaamen 2010).

Previous studies have suggested that lysoPCs are pro-inflammatory and pro-atherogenic metabolites that participate in monocyte adhesion (Weber *et al.*

1995), smooth muscle cell migration and proliferation (Kume and Gimbrone Jr 1994) as well as in endothelium-dependent vasodilation impairment (Chen *et al.* 1997). In contrast, recent large-scale prospective studies reported inverse associations between circulating lysoPCs (e.g. 16:0, 18:0, 18:1, 18:2), CAD and total mortality (Ganna *et al.* 2014; Meikle *et al.* 2011; Sigruener *et al.* 2014; Stegemann *et al.* 2014). In patients with type 2 diabetes, lysoPC a C18:2 associated negatively with both incident diabetes and impaired glucose tolerance (Wang-Sattler *et al.* 2012). Another study suggested that CVD development is preceded by reduced plasma levels of lysoPC a C16:0 and lysoPC a C20:4 and showed an inverse correlation between lysoPC a C16:0 and carotid IMT (Fernandez *et al.* 2013).

The above-mentioned findings seem counterintuitive, since the production of lysoPCs depends largely on pro-atherogenic lipoprotein-associated phospholipase A2. Furthermore, elevated lysoPC content in atherosclerotic plaques has also been reported (Stegemann *et al.* 2011). However, one can hypothesize that lower circulating lysoPC levels reflect their increased catabolism and more efficient removal from blood into tissues (Croset *et al.* 2000; Stegemann *et al.* 2014), either in the form of oxLDL, or directly from albumin (Meikle *et al.* 2011). Moreover, lower activity of lecithin-cholesterol acyltransferase may also partially explain reduced circulating lysoPC levels in atherosclerotic patients (Duivenvoorden *et al.* 2011; Rasmiena *et al.* 2013). Thus, the pro- or antiatherogenicity of a lysoPC molecule seems to be dependent on the physical properties of its fatty acid residue (e.g. 16:0, 18:1, 20:4).

In the current thesis, the serum levels of various lysoPCs were determined and their possible relationship with arterial stiffness, hemodynamics, ED and inflammation in CAD patients, PAD patients and in clinically healthy subjects was assessed (Papers III and IV).

3. AIMS OF THE THESIS

The general aim of the present thesis was to determine the metabolomic profile of arterial stiffness and the role of early biomarkers of renal damage in patients with atherosclerosis and in clinically healthy subjects.

Specific aims

1. To measure the serum levels of fibulin-1 (FBLN-1) and their association with arterial stiffness and inflammation in non-diabetic coronary artery disease patients, peripheral arterial disease patients and in clinically healthy controls.
2. To determine the serum levels of neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (CysC) and the urinary concentrations of liver-type fatty acid-binding protein (L-FABP) and kidney injury molecule-1 (KIM-1), as well as to evaluate their association with arterial stiffness and inflammation in coronary artery disease patients without moderate to severe chronic kidney disease (estimated glomerular filtration rate ≥ 60 mL/min/1.73 m²) and in clinically healthy subjects.
3. To investigate the relationship of aortic stiffness, hemodynamics and inflammation with serum acylcarnitines in patients with coronary artery disease and in clinically healthy controls.
4. To study serum phosphatidylcholine and lysophosphatidylcholine species in relation to arterial stiffness, hemodynamics and endothelial dysfunction in coronary artery disease patients, peripheral arterial disease patients and in clinically healthy subjects.
5. To observe and analyze similarities and differences in arterial function and in metabolomic and inflammatory profiles between patients with coronary artery disease and patients with peripheral arterial disease.

4. SUBJECTS AND METHODS

4.1. Study population

4.1.1. Patients with symptomatic coronary artery disease and patients with symptomatic peripheral arterial disease

A total of 90 male patients, including 52 subjects with stable CAD (n=38, n=52, n=52, n=52 in Papers I–IV, respectively) and 38 subjects with symptomatic PAD (n=38 and n=32 in Papers I and IV, respectively), were recruited from the Department of Cardiology and from the Department of Vascular Surgery, Tartu University Hospital, Estonia. The diagnoses of CAD and PAD were established based on significant stenoses ($\geq 50\%$ diameter reduction) or occlusions of arteries confirmed by coronary angiography and digital subtraction angiography of the abdominal aorta and lower extremities, respectively. The PAD patients fell into four stages according to the Fontaine: stage II = intermittent claudication (n=24 and n=22 in Papers I and IV, respectively); stage III = leg pain at rest (n=6 and n=3 in Papers I and IV, respectively); stage IV = focal tissue necrosis or gangrene (n=8 and n=7 in Papers I and IV, respectively). The exclusion criteria for both patient groups were any comorbid acute or chronic inflammatory disease, diabetes mellitus (fasting serum glucose level >7 mmol/L), MI, cerebrovascular events or revascularization operation during the preceding 6 months, unstable angina, cardiac arrhythmias, clinically significant heart failure or valvular disease, reduced kidney function (eGFR <60 ml/min/1.73 m²), presence of cancer or endocrine pathology. In Papers II and IV, 6 (16%) PAD patients with CAD as the comorbidity were included in analysis. CAD patients with symptomatic PAD were not recruited.

4.1.2. Clinically healthy subjects

In total, 41 age- and gender-matched clinically healthy controls (n=30, n=41, n=40 and n=40 in Papers I–IV, respectively) were identified through local family physicians in the same geographical area. The following exclusion criteria were set: any comorbid acute or chronic inflammatory disease, CAD, cerebral or peripheral atherosclerotic disease, diabetes mellitus, cardiac arrhythmias, clinically significant heart failure or valvular disease, reduced kidney function (eGFR <60 ml/min/1.73 m²), presence of cancer, infectious disease or endocrine pathology. None of the subjects was on regular medication.

4.2. Study design and protocol

All subjects completed a lifestyle and medical history questionnaire. Venous blood samples were drawn from each subject (Papers I–IV) and spot morning urine specimens were collected from 45 CAD patients and 37 controls (Paper

II). All samples were obtained between 8am and 11am after an overnight fast and abstinence from tobacco, alcohol and caffeine-containing beverages. Height and weight were assessed and body mass index (BMI) was calculated. The subjects were studied after 10 minutes of rest in a supine position in a quiet, temperature-controlled room. Thereafter, brachial BP, cf-PWV and cr-PWV (Paper I) were assessed and PWA was made. All hemodynamic measurements were taken in duplicate and averaged. Each subject gave his written informed consent, and the study protocol was approved by the Ethics Committee of the University of Tartu.

4.3. Methods

4.3.1. Biochemical analysis of blood and urine

Peripheral venous blood samples were collected in serum separator tubes (BD SST™ II Advance) for biochemical analysis (Papers I–IV). The serum levels of glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, white blood cell (WBC) count, PLT count, hsCRP, CysC, urea, creatinine (eGFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation (Levey et al. 2009)), and urinary creatinine were measured in the local clinical laboratory with automated analyzers using standard laboratory methods.

Other blood samples were centrifuged within 30 minutes of collection and the serum was pipetted into Eppendorf tubes. The tubes were stored at $-70\text{ }^{\circ}\text{C}$ until assayed. The serum levels of IL-6, ferritin, PAI-1, resistin and insulin were determined using The Evidence Investigator (Metabolic Syndrome Array-1, Randox Laboratories, Crumlin, UK). Serum FBN-1, NGAL, MPO, oxLDL and adiponectin levels were measured using an enzyme-linked immunosorbent assay (ELISA) (Human Fibulin-1 Kit, Cusabio Biotech Co., Ltd; Human Lipocalin-2/NGAL immunoassay, R&D Systems Europe, Abingdon, UK; Myeloperoxidase enzyme immunoassay test kit, BIOCHECK, Inc. Foster City, CA; Merckodia Oxidized LDL ELISA, Uppsala, Sweden; Human Total Adiponectin/Acrp30 immunoassay, R&D Systems Europe, Abingdon, UK, respectively).

Urine samples were collected in sterile plastic collection cups, aliquoted into Eppendorf tubes and kept frozen at $-70\text{ }^{\circ}\text{C}$ until analysis. The levels of urinary L-FABP and KIM-1 were measured using ELISA (Human Liver Fatty Acid Binding Protein-1 ELISA kit, BlueGene Biotech, Shanghai, China; HAVCR1 (Human), KIM-1, Abnova GmbH, Heidelberg, Germany, respectively).

4.3.2. Targeted serum metabolite profiling

Peripheral venous blood samples were collected in plain tubes (Plain BD Vacutainer® Tubes) for metabolomic analysis (Papers III and IV). All samples were centrifuged within 60 minutes of collection and the supernatant was transferred into Eppendorf tubes. The tubes were frozen at $-70\text{ }^{\circ}\text{C}$ until assayed.

The serum levels of metabolites were determined with the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) using the flow injection analysis tandem mass spectrometry as well as liquid chromatography techniques. All measurements were performed as described in the manufacturer's manual UM-P180. The AbsoluteIDQ™ p180 kit allows simultaneous quantification of 186 metabolites (acylcarnitines (Cx:y), amino acids and biogenic amines, hexose, sphingolipids (SM x:y or SM (OH) x:y), glycerophospholipids (lysoPCs (lysoPC a x:y) and PCs (PC aa x:y and PC ae x:y)) from 10 µL of serum (Table 1). Lipid side-chain composition is abbreviated as Cx:y, where x denotes the number of carbons in the side chain and y denotes the number of double bonds. Glycerophospholipids are differentiated further according to the presence of ester and ether bonds in the glycerol moiety. Double letters (aa=diacyl, ae=acyl-alkyl) indicate that two glycerol positions are bound to a fatty acid residue, while a single letter (a=acyl or e=alkyl) indicates a bond with only one fatty acid residue. Identification and quantification of the metabolites was achieved using multiple reaction monitoring along with internal standards. The concentrations of all metabolites were automatically calculated in µM by the MetIDQ™ software (BIOCRATES Life Sciences AG). Metabolites below the limit of detection (n=35 and n=7 in Papers III and IV, respectively), determined experimentally by BIOCRATES, were excluded from further analysis. The assay procedures of the AbsoluteIDQ™ p180 kit have been described in detail previously (Nkuipou-Kenfack *et al.* 2014).

Table 1. Low molecular weight metabolites that were quantified using the AbsoluteIDQ® p180 kit (http://www.biocrates.com/images/ListofMetabolites_p180.pdf) in our study.

ACYLCARNITINES			
C0	Carnitine	C10:1	Decenoylcarnitine
C2	Acetylcarnitine	C10:2	Decadienylcarnitine
C3	Propionylcarnitine	C12	Dodecanoylcarnitine
C3:1	Propenoylcarnitine	C12:1	Dodecenoylcarnitine
C3-OH	Hydroxypropionylcarnitine	C12-DC	Dodecanedioylcarnitine
C4	Butyrylcarnitine	C14	Tetradecanoylcarnitine
C4:1	Butenylcarnitine	C14:1	Tetradecenoylcarnitine
C4-OH (C3-DC)	Hydroxybutyrylcarnitine	C14:1-OH	Hydroxytetradecenoylcarnitine
C5	Valerylcarnitine	C14:2	Tetradecadienylcarnitine
C5:1	Tiglylcarnitine	C14:2-OH	Hydroxytetradecadienylcarnitine
C5:1-DC	Glutaconylcarnitine	C16	Hexadecanoylcarnitine
C5-DC (C6-OH)	Glutaryl carnitine (Hydroxyhexanoylcarnitine)	C16:1	Hexadecenoylcarnitine
C5-M-DC	Methylglutaryl carnitine	C16:1-OH	Hydroxyhexadecenoylcarnitine
C5-OH (C3-DC-M)	Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	C16:2	Hexadecadienylcarnitine
C6 (C4:1-DC)	Hexanoylcarnitine (Fumaryl carnitine)	C16:2-OH	Hydroxyhexadecadienylcarnitine
C6:1	Hexenoylcarnitine	C16-OH	Hydroxyhexadecanoylcarnitine
C7-DC	Pimelylcarnitine	C18	Octadecanoylcarnitine
C8	Octanoylcarnitine	C18:1	Octadecenoylcarnitine
C9	Nonaylcarnitine	C18:1-OH	Hydroxyoctadecenoylcarnitine
C10	Decanoylcarnitine	C18:2	Octadecadienylcarnitine

Table 1. Continued

AMINO ACIDS AND BIOGENIC AMINES			
Ala	Alanine	Ac-Orn	Acetylnornithine
Arg	Arginine	ADMA	Asymmetric dimethylarginine
Asn	Asparagine	SDMA	Symmetric dimethylarginine
Asp	Aspartate	alpha-AAA	alpha-Amino adipic acid
Cit	Citrulline	Carnosine	Carnosine
Gln	Glutamine	Creatinine	Creatinine
Glu	Glutamate	Histamine	Histamine
Gly	Glycine	Kynurenine	Kynurenine
His	Histidine	Met-SO	Methioninesulfoxide
Ile	Isoleucine	Nitro-Tyr	Nitrotyrosine
Leu	Leucine	cis-OH-Pro	cis-4-Hydroxyproline
Lys	Lysine	trans-OH-Pro	trans-4-Hydroxyproline
Met	Methionine	PEA	Phenylethylamine
Orn	Ornithine	Putrescine	Putrescine
Phe	Phenylalanine	Sarcosine	Sarcosine
Pro	Proline	Serotonin	Serotonin
Ser	Serine	Spermidine	Spermidine
Thr	Threonine	Spermine	Spermine
Trp	Tryptophan	Taurine	Taurine
Tyr	Tyrosine	Dopamine	Dopamine
Val	Valine	DOPA	DOPA
MONOSACCHARIDES	GLYCEROPHOSPHOLIPIDS		
Sum of Hexoses	lysoPC a C16:0	PC aa C36:3	PC ae C34:2
	lysoPC a C16:1	PC aa C36:4	PC ae C34:3
SPHINGOLIPIDS	lysoPC a C17:0	PC aa C36:5	PC ae C36:0
	lysoPC a C18:0	PC aa C36:6	PC ae C36:1
SM (OH) C14:1	lysoPC a C18:1	PC aa C38:0	PC ae C36:2
SM C16:0	lysoPC a C18:2	PC aa C38:1	PC ae C38:3
SM C16:1	lysoPC a C20:3	PC aa C38:3	PC ae C38:4
SM (OH) C16:1	lysoPC a C20:4	PC aa C38:4	PC ae C38:5
SM C18:0	lysoPC a C24:0	PC aa C38:5	PC ae C38:6
SM C18:1	lysoPC a C26:0	PC aa C38:6	PC ae C40:1
SM C20:2	lysoPC a C26:1	PC aa C40:1	PC ae C40:2
SM C22:3 1) 2)	lysoPC a C28:0	PC aa C42:1	PC ae C40:3
SM (OH) C22:1	lysoPC a C28:1	PC aa C42:2	PC aa C40:4
SM (OH) C22:2	PC aa C24:0	PC aa C42:4	PC ae C40:5
SM C24:0	PC aa C26:0	PC aa C42:5	PC ae C40:6
SM C24:1	PC aa C28:1	PC aa C42:6	PC ae C42:0
SM (OH) C24:1	PC aa C30:0	PC ae C30:0	PC ae C42:1
SM C26:0	PC aa C34:2	PC ae C30:1	PC ae C42:2
SM C26:1	PC aa C34:3	PC ae C30:2	PC ae C42:3
	PC aa C34:4	PC ae C32:1	PC ae C42:4
	PC aa C36:0	PC ae C32:2	PC ae C42:5
	PC aa C36:1	PC ae C34:0	PC ae C44:3
	PC aa C36:2	PC ae C34:1	

Abbreviations: a, acyl; aa, diacyl; ae, acyl-alkyl; lysoPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin.

4.3.3. Peripheral blood pressure measurement

Brachial BP was measured using an automatic oscillometric BP monitor (OMRON M4-I; Omron Healthcare Europe, Hoofddorp, the Netherlands) following 10 minutes of supine rest (Papers I–IV). The MAP was calculated from integration of the radial pressure waveform using the Sphygmocor software (SCOR® Px, 7.0; AtCor Medical, Sydney, Australia). The difference between peripheral systolic BP (PSBP) and peripheral diastolic BP (PDBP) was expressed as PPP. The PPP/PP (Paper I) was defined as the percentage ratio of PPP to PP (the difference between CSBP and CDBP). All BP measurements were taken in duplicate and the mean values were used in subsequent analyses.

4.3.4. Assessment of arterial stiffness and central hemodynamics

4.3.4.1. Pulse wave analysis

Central hemodynamic parameters, namely CSBP, CDBP, CPP, travel time of the reflected wave and central AIx were evaluated non-invasively by PWA (Papers I–IV), using the SphygmoCor device (version 7.1, AtCor Medical, Sydney, Australia). At least 15 sequential high-quality radial artery pressure waveforms were recorded at the patient's left wrist using a high-fidelity micro-manometer (SPT-301B; Millar Instruments, USA). Thereafter, a validated generalized transfer function for calculation of the central aortic pressure waveform was employed (Adji *et al.* 2011; Pauca *et al.* 2001). The AIx was defined as the difference between the second and first systolic peaks of the central aortic wave, expressed as a percentage of CPP (Laurent *et al.* 2006). It was corrected for a heart rate of 75 beats per minute (AIx@75) by a Sphygmocor built-in algorithm.

4.3.4.2. Pulse wave velocity

The PWV was obtained by sequential recordings of the carotid and femoral (cf-PWV in Papers I–IV) or radial (cr-PWV in Paper I) pulse waveforms (SphygmoCor Px, version 7.1, AtCor Medical, Australia) using an arterial tonometer (SPT-301B, Millar Instruments, USA). The R wave of the ECG signal serves as the reference in determining the time it takes a pulse wave to travel between two measuring sites. The PWV (m/s) was calculated as a ratio between traveled distance and transit time ($PWV = \text{distance}/\text{time}$) (Van Bortel *et al.* 2012). The tape measure distance from the suprasternal notch over the umbilicus to the femoral artery minus carotid arterial length was used for the calculation of cf-PWV; to calculate cr-PWV, the distance from the suprasternal notch to the radial artery minus carotid arterial length was required. Recordings where heart rate varied more than 5 beats per minute were excluded. All PWV measurements were taken in duplicate and the mean values were used in subsequent analysis.

4.3.5. Measurement of angiographic score

In patients with PAD, digital subtraction angiography (Axiom Artis; Siemens Medical Solutions, Forchheim, Germany) of the aorta and lower extremity arteries was performed using a femoral approach at the Department of Radiology, Tartu University Hospital, Estonia. The angiographic score for this vascular region (Nylaende *et al.* 2006) was determined by an experienced radiologist, who was blinded to all other data. For the 21-segment model, the following arterial segments were evaluated: abdominal aorta, common iliac artery, external iliac artery, common femoral artery, profunda femoris artery, superficial femoral artery, popliteal artery, tibio-peroneal trunk, anterior tibial artery, posterior tibial artery and peroneal artery. Severity of luminal narrowing was graded as follows: 0 = normal; 1 = stenosis <50%; 2 = stenosis >50%; 3 = occlusion (Nylaende *et al.* 2006). A score was assigned only to the area with the most severe stenosis in each segment. These scores were then summed to obtain the overall angiographic score (maximum 63) (Paper IV).

4.3.6. Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences software for Windows, version 22.0 (Chicago, IL, USA). Continuous variables are expressed as mean±standard deviation or as median and interquartile ranges. Categorical variables are expressed in percentages. The normality of the distribution of continuous variables was assessed using the Shapiro-Wilk test (Papers I–IV), and the skewed distributions were normalized by logarithmic transformation.

Comparisons between the two independent groups were performed using two-tailed Student's t-test for normally distributed data and the Mann-Whitney U test for non-normal data. One-way analysis of variance was employed for comparing the mean values of normally distributed variables among the three unpaired groups, and post hoc analysis was performed by the Scheffé test (Scheffé 1959) to check for intergroup differences (Papers I and IV). A Kruskal-Wallis test was conducted to determine differences between the median values of non-normally distributed variables among the three unpaired groups and pairwise comparisons were performed using Dunn's procedure (Dunn 1964) with a Bonferroni correction for multiple comparisons (Papers I and IV). For the dichotomous measures, Fisher's exact test or chi-square test was employed to compare two groups for each variable (Papers II and III), and the Fisher-Freeman-Halton test was conducted when the three groups were compared (Papers I and IV).

Pearson's correlation coefficient and Spearman's rank correlation coefficient were used to measure the strength of the relationship between normally and non-normally distributed continuous variables, respectively. Multiple regression analysis was applied to investigate the independent determinants of AIx@75 (Paper I), FBLN-1 (Paper I), cf-PWV (Papers II–IV), heart rate (Paper IV),

ADMA (Paper IV) and ADMA/Arg (Paper IV). Explanatory power of the regression models was estimated with adjusted R^2 values.

Analysis of covariance was used as follows: 1) to adjust cf-PWV (Papers I–IV) and cr-PWV (Paper I) for MAP, 2) to adjust low molecular weight metabolites for BMI and statin use (Paper III), and 4) to adjust low molecular weight metabolites for BMI, statin use and smoking status (Paper IV).

Principal component analysis (PCA) was employed to reduce the large number of correlated metabolites to fewer uncorrelated factors (Paper III). To identify interpretable factors, Varimax rotation was carried out; only the factors that explained at least 5% of total variance were retained. Also, only the metabolites with absolute factor loadings ≥ 0.60 were reported as the components of a given factor in order to avoid false discoveries. Thereafter, for each subject, factor scores (weighted sum of the standardized metabolites within that factor, weighted on the factor loading for each individual metabolite) were calculated and included in the subsequent analyses.

Statistical significance was defined as $p < 0.05$ (Papers I–IV). To account for multiple testing the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995) was used to control false discovery rate at the level of 0.05 (Papers III–IV).

5. RESULTS

5.1. Relationships between serum fibulin-1, arterial stiffness and inflammation in patients with atherosclerosis and in healthy subjects (Paper I)

Characteristics of the study population

The baseline characteristics of the study groups are presented in Table 2. There were no significant differences in mean age, PDBP, CDBP, total and LDL cholesterol, oxLDL, PAI-1 or resistin levels between the investigated groups. The patients with PAD differed significantly from both the CAD and control subjects with respect to height, PSBP, CSBP, PPP/PPP, WBC count, HDL cholesterol, hsCRP, AIx@75 and current smoking status. Differences in MAP, heart rate, cf-PWV, PLT count, triglycerides and FBLN-1 levels only emerged from comparison to the controls. The CAD patients had significantly higher BMI and glucose levels than the PAD patients and higher cr-PWV and WBC count than the controls. Medication use among the three study groups is presented in Table 3.

Association between arterial stiffness and biochemical markers

In univariate analysis, FBLN-1 correlated positively with AIx@75 for the PAD patients ($\rho=0.37$, $p=0.021$; Figure 2) (this relationship retained significance even after excluding outliers, $r=0.47$, $p=0.005$), but not for the CAD patients ($\rho=-0.02$, $p=0.91$) or for the control group ($r=0.01$, $p=0.95$). In multivariate analysis with AIx@75 as the dependent variable, FBLN-1, MAP and height were significant independent variables in the PAD group ($R^2=0.45$; $p<0.001$; Table 4). There was no correlation between serum FBLN-1 and cf-PWV in any of the studied groups (data not shown).

In univariate analysis, FBLN-1 was correlated with resistin ($\rho=0.40$; $p=0.013$), WBC count ($\rho=0.39$; $p=0.018$) and PLT count ($\rho=0.38$; $p=0.019$) for the PAD group, while in multivariate analysis with FBLN-1 as the dependent variable, resistin and AIx@75, remained significant determinants for these patients ($R^2=0.31$, $p=0.002$; Table 5). In the CAD group, WBC count ($\rho=0.36$, $p=0.035$) and height ($\rho=-0.41$, $p=0.012$) correlated univariately with FBLN-1 and remained significant in multivariate analysis ($R^2=0.33$, $p=0.002$; Table 5), while for the control subjects, only PAI-1 was found to correlate with FBLN-1 ($r=0.39$; $p=0.048$). However, this difference did not reach statistical significance in multivariate analysis (data not shown).

Table 2. Baseline characteristics of the study groups.

Variable	PAD patients (n=38)	CAD patients (n=38)	Controls (n=30)	p-value
Age (years)	62.4 ± 9.0	64.0 ± 9.5	61.1 ± 6.4	0.36
BMI (kg/m ²)	25.5 ± 3.3 ^b	27.5 ± 3.2	26.0 ± 3.3	0.02
Height (cm)	172.0 ± 5.8 ^{a,b}	175.8 ± 6.6	179.2 ± 6.5	<0.001
Peripheral SBP (mmHg)	144 ± 18 ^{a,b}	135 ± 15	127.0 ± 13	<0.001
Peripheral DBP (mmHg)	81 ± 9	78 ± 8	77 ± 7	0.20
Central SBP (mmHg)	135 ± 17 ^{a,b}	124 ± 13	118 ± 13	0.004
Central DBP (mmHg)	81 ± 10	79 ± 7	78 ± 7	0.25
MAP (mmHg)	103 ± 12 ^a	97 ± 9	95 ± 10	0.004
Heart rate (bpm)	64.6 ± 11.3 ^a	61.4 ± 7.8	57.6 ± 7.3	0.008
PPP/PPP	1.19 ± 0.9 ^{a,b}	1.28 ± 0.1	1.28 ± 0.1	0.001
WBC count (x10 ⁹ /L)	7.8 ± 1.9 ^{a,b}	6.3 ± 1.6 ^a	4.9 ± 0.6	<0.001
PLT count (x10 ⁹ /L)	244 ± 59 ^a	219 ± 48	213 ± 31	0.03
Total cholesterol (mmol/L)	5.4 ± 1.2	5.2 ± 1.1	5.5 ± 1.0	0.41
HDL cholesterol (mmol/L)	1.1 (1.0–1.4) ^{a,b}	1.5 (1.1–1.7)	1.5 (1.3–1.8)	<0.001
LDL cholesterol (mmol/L)	3.6 ± 1.1	3.4 ± 1.1	3.6 ± 0.9	0.54
Triglycerides (mmol/L)	1.6 (0.9–2.2) ^a	1.2 (0.8–1.7)	0.9 (0.7–1.4)	0.01
Glucose (mmol/L)	5.4 ± 0.8 ^b	5.8 ± 0.6	5.8 ± 0.5	0.02
eGFR (ml/min/1.73 m ²)	91.1 ± 18.4	82.8 ± 14.4	83.4 ± 11.6	0.04
hsCRP (mg/L)	4.4 (1.1–7.6) ^{a,b}	1.3 (0.8–2.6)	1.1 (0.7–2.0)	<0.001
oxLDL (U/L)	67.5 (53.4–85.6)	61.0 (50.3–81.8)	66.3 (53.5–84.4)	0.63
PAI-1 (ng/ml)	28.0 ± 9.3	26.4 ± 8.7	27.3 ± 6.6	0.73
Resistin (ng/ml)	3.5 (2.8–4.5)	3.7 (2.8–4.9)	2.8 (2.4–3.4)	0.25
Fibulin-1 (µg/ml)	9.4 (4.9–17.8)^a	7.1 (4.8–11.8)	5.6 (4.1–8.4)	0.005
AIx@75 (%)	29.4 ± 7.2 ^{a,b}	19.2 ± 7.2	15.4 ± 7.1	<0.001
cf-PWV (m/s) ^c	9.8 ± 2.2 ^a	9.5 ± 2.2	8.3 ± 2.2	0.023
cr-PWV (m/s) ^c	9.3 ± 1.2	9.5 ± 1.1 ^a	8.7 ± 1.2	0.024
Current smoking, n (%)	28 (72) ^{a,b}	8 (21)	3 (10)	<0.001

^a = <0.05 vs control group

^b = <0.05 vs CAD group

^c = cf-PWV and cr-PWV have been adjusted for MAP.

The values are represented as means (± standard deviation), medians (inter-quartile range), or prevalence (%). AIx@75, augmentation index adjusted to a heart rate of 75 beats per minute; BMI, body mass index; CAD, coronary artery disease; cf-PWV, carotid–femoral pulse wave velocity; cr-PWV, carotid–radial pulse wave velocity; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MAP, mean arterial pressure; PAD, peri-

pheral arterial disease; PAI-1, plasminogen activator inhibitor-1; PLT, platelet; PPP/PP, pulse pressure amplification; SBP, systolic blood pressure; WBC, white blood cell.

Table 3. Medication use among the three study groups.

	PAD patients (n=38)	CAD patients (n=38)	Controls (n=30)	p-value
Medication, n (%)				
ACE inhibitors	9 (23) ^a	13 (34) ^a	0 (0)	<0.001
ARBs	6 (15)	6 (16)	0 (0)	0.04
Calcium channel blockers	6 (15) ^b	14 (37) ^a	0 (0)	<0.001
Beta blockers	10 (26) ^{a,b}	27 (71) ^a	0 (0)	<0.001
Diuretics	3 (8)	4 (11)	0 (0)	0.20
Pentoxifylline	13 (33) ^{a,b}	0 (0)	0 (0)	<0.001
Naftidrofuryl	8 (21) ^{a,b}	0 (0)	0 (0)	<0.001
Antiplatelets	13 (33) ^{a,b}	26 (68) ^a	0 (0)	<0.001
Statins	7 (18) ^{a,b}	20 (53) ^a	0 (0)	<0.001

^a = <0.05 vs control group

^b = <0.05 vs CAD group

The values are represented as prevalence (%). ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

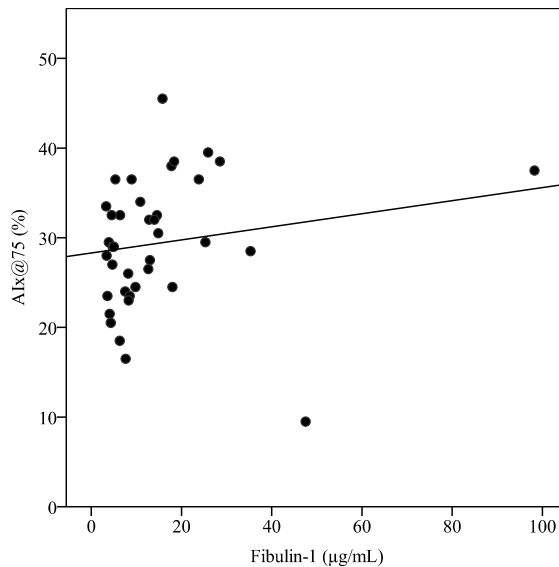


Figure 2. Scatterplot showing correlation between heart rate-corrected augmentation index (AIx@75) and serum fibulin-1 for the peripheral arterial disease patients ($\rho=0.37$, $p=0.021$).

Table 4. Multiple regression model for PAD group with AIx@75 as the dependent variable.

Variable	Regression coefficient	Standard error	<i>p</i> -value
FBLN-1	0.477	0.117	<0.001
MAP	0.222	0.069	0.003
Height	-0.298	0.140	0.041

$R^2=0.454$, $p<0.001$. AIx@75, augmentation index adjusted to a heart rate of 75 beats per minute; FBLN-1, fibulin-1; MAP, mean arterial pressure; PAD, peripheral arterial disease.

Table 5. Multiple regression model for PAD and CAD patients with FBLN-1 as the dependent variable.

Variable	Regression coefficient	Standard error	<i>p</i> -value
<i>PAD patients^a</i>			
AIx@75	0.495	0.160	0.004
Resistin	1.523	0.719	0.042
Antihypertensive treatment	-2.710	2.046	0.195
<i>CAD patients^b</i>			
WBC count	0.875	0.328	0.013
Height	-0.210	0.081	0.015
Statin treatment	-1.933	1.095	0.089

^a $R^2=0.314$, $p=0.002$; ^b $R^2=0.332$, $p=0.002$. AIx@75, augmentation index adjusted to a heart rate of 75 beats per minute; CAD, coronary artery disease; FBLN-1, fibulin-1; PAD, peripheral arterial disease; WBC, white blood cell.

5.2. Early biomarkers of renal damage in relation to arterial stiffness and inflammation in coronary artery disease patients and in healthy subjects (Paper II)

Characteristics of the study population

The clinical and laboratory characteristics of the CAD cases and the controls are presented in Table 6. There were no significant differences between the groups with respect to mean age, PDBP, CSBP, CDBP, MAP, current smoking status, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, hsCRP or ferritin levels. The two groups differed in BMI, height, PSBP, heart rate, cf-PWV, AIx@75, medication use, as well as in WBC count and serum MPO, IL-6, adiponectin and resistin levels. There were no significant differences in eGFR, serum creatinine, urea, CysC, NGAL, urinary L-FABP or KIM-1 levels between the patients and the controls (Table 7).

Association between arterial stiffness and markers of renal function and tubular damage

In a univariate analysis for the predefined variables, log L-FABP ($r=0.44$, $p=0.002$; Figure 3), CysC ($r=0.52$, $p<0.001$), eGFR ($\rho=-0.36$, $p=0.012$) and creatinine ($r=0.33$, $p=0.021$) correlated positively with cf-PWV for the CAD group but not for the controls (data not shown). Also, log KIM-1 showed a borderline significant relationship ($r=0.29$, $p=0.062$) with aortic stiffness for the patients. In multivariate analysis with cf-PWV as the dependent variable, L-FABP and KIM-1 were the only renal markers that remained significant after adjustment for potential confounders (mean age, BMI, MAP, NGAL, eGFR, hsCRP, IL-6, glucose, cholesterol, triglycerides, smoking status, antihypertensive therapy and statin use) ($R^2=0.58$; $p<0.001$; Table 8). In the post hoc subgroup analyses based on eGFR levels, L-FABP ($r=0.6$, $p=0.002$) showed a strong and CysC ($r=0.4$, $p=0.043$) a moderate correlation with cf-PWV for the subgroup of CAD patients with eGFR between 60 and 89 mL/min/1.73 m².

Serum NGAL was not correlated with cf-PWV or any other measure of arterial stiffness for either CAD patients or controls (data not shown).

In the control group, a significant inverse correlation was found between serum resistin levels and log L-FABP ($r=-0.34$, $p=0.045$). Serum NGAL levels correlated with WBC count ($\rho=0.29$, $p=0.038$; $r=0.35$, $p=0.029$) and resistin ($\rho=0.60$, $p<0.001$; $r=0.57$, $p<0.001$) for the CAD and control groups, respectively, and with serum creatinine ($\rho=0.31$, $p=0.030$) only for the CAD group. Urinary log KIM-1 was linearly correlated with serum creatinine ($r=0.33$, $p=0.019$), adiponectin ($r=0.31$, $p=0.028$) and log ferritin ($r=-0.32$, $p=0.022$) levels for the CAD group while no such correlations were found for the controls.

Table 6. Baseline characteristics of the study groups.

Variable	CAD patients ($n=52$)	Controls ($n=41$)	<i>p</i> -value
Age (years)	63.2 ± 9.2	60.1 ± 7.2	0.075
Body mass index (kg/m ²)	27.9 ± 3.5	26.0 ± 3.4	0.012
Height (cm)	176.2 ± 6.4	179.3 ± 6.5	0.025
Peripheral SBP (mmHg)	135.0 ± 14.5	128.4 ± 13.2	0.027
Peripheral DBP (mmHg)	78.5 ± 8.0	77.9 ± 7.3	0.706
Central SBP (mmHg)	122.7 ± 11.1	119.8 ± 15.1	0.303
Central DBP (mmHg)	79.7 ± 7.6	78.4 ± 6.7	0.378
MAP (mmHg)	96.0 (91.6–101.4)	92.5 (89.5–99.5)	0.103
Heart rate (bpm)	62.8 ± 8.3	57.8 ± 7.1	0.003
WBC count (x10 ⁹ /L)	6.4 (5.2–7.9)	5.1 (4.7–5.8)	0.001
Total cholesterol (mmol/L)	5.1 ± 1.0	5.5 ± 0.9	0.080
HDL cholesterol (mmol/L)	1.5 ± 0.4	1.6 ± 0.4	0.153

Table 6. Continued

Variable	CAD patients (n=52)	Controls (n=41)	p-value
LDL cholesterol (mmol/L)	3.2 (2.8–4.1)	3.7 (3.3–4.3)	0.058
Triglycerides (mmol/L)	1.2 (0.8–1.6)	1.0 (0.7–1.4)	0.311
Glucose (mmol/L)	5.8 (5.3–6.3)	5.8 (5.4–6.1)	0.858
hsCRP (mg/L)	1.3 (0.7–2.8)	1.2 (0.7–2.1)	0.423
Myeloperoxidase (ng/ml)	58.4 (35.3–110.7)	45.2 (28.0–62.6)	0.014
Interleukin-6 (pg/ml)	1.33 (0.74–1.98)	0.95 (0.64–1.29)	0.048
Ferritin (ng/ml)	128.0 (68.6–202.0)	92.7 (54.7–190.7)	0.110
Adiponectin (ng/ml)	5701 ± 2890	7081 ± 3612	0.045
Resistin (ng/ml)	3.4 (2.6–4.5)	2.8 (2.4–3.5)	0.043
AIx@75 (%)	18.9 ± 7.2	15.1 ± 8.0	0.021
cf-PWV (m/s)	9.7 ± 2.6	8.2 ± 1.7	0.003
Current smoking, n (%)	13 (25)	5 (12)	0.214
Medication, n (%)			
Antihypertensives	48 (92)	0 (0)	<0.001
Antiplatelets	34 (65)	0 (0)	<0.001
Statins	28 (54)	0 (0)	<0.001

The values are expressed as means (\pm standard deviation), medians (interquartile range), or prevalence (%). AIx@75, augmentation index adjusted to a heart rate of 75 beats per minute; CAD, coronary artery disease; cf-PWV, carotid–femoral pulse wave velocity; DBP, diastolic blood pressure; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MAP, mean arterial pressure; SBP, systolic blood pressure; WBC, white blood cell.

Table 7. Biomarkers of renal function and damage.

Variable	CAD patients (n=52)	Controls (n=41)	p-value
eGFR (ml/min/1.73 m ²)	85 (73–97)	89 (79–93)	0.847
S-Creatinine (μ mol/L)	83.2 ± 12.3	84.0 ± 11.2	0.750
S-Urea (mmol/L)	5.7 (4.7–7.0)	5.3 (4.5–6.0)	0.114
Cystatin C (mg/L)	0.93 ± 0.14	0.90 ± 0.10	0.278
NGAL (ng/ml)	74.6 (61.9–83.5)	73.7 (59.4–85.0)	0.892
L-FABP/Creat (ng/mmol)	169.0 (121.9–228.2)	187.2 (136.5–271.3)	0.244
KIM-1/Creat (ng/mmol)	79.6 (53.6–110.7)	85.8 (54.7–118.5)	0.636

The values are expressed as means (\pm standard deviation) or medians (inter-quartile range). CAD, coronary artery disease; Creat, creatinine; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein; NGAL, neutrophil gelatinase-associated lipocalin.

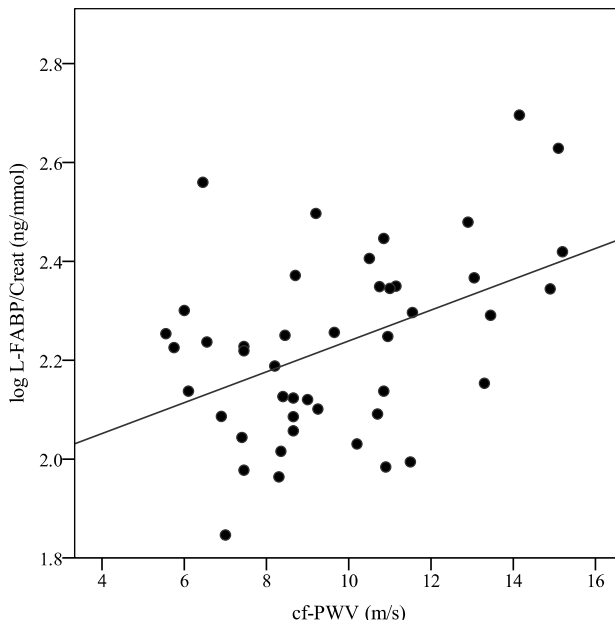


Figure 3. Scatterplot showing linear correlation between carotid-femoral pulse wave velocity (cf-PWV) and logarithmically transformed urinary liver-type fatty acid-binding protein (log L-FABP) for the coronary artery disease patients ($r=0.44$, $p=0.002$).

Table 8. Multiple regression model for the CAD group with cf-PWV as the dependent variable.

Variable	Regression coefficient	Standard error	p -value
Mean age	0.161	0.029	<0.001
L-FABP/Creat	0.011	0.003	0.001
Body mass index	0.197	0.077	0.014
KIM-1/Creat	0.013	0.006	0.023

$R^2=0.584$, $p<0.001$. CAD, coronary artery disease; cf-PWV, carotid-femoral pulse wave velocity; Creat, creatinine; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein.

5.3. Serum short- and medium-chain acylcarnitines independently determine aortic stiffness in coronary artery disease patients (Paper III)

Characteristics of the study population

Table 9 summarizes the hemodynamic and biochemical characteristics of the two study groups. The groups did not differ significantly from each other with respect to mean age, PSBP, PDBP, CSBP, CDBP, MAP, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, oxLDL, glucose or hsCRP levels. Significant differences between the groups were found in BMI, height, heart rate, cf-PWV and AIx@75, as well as in eGFR, WBC count and serum MPO, IL-6 and adiponectin levels.

Table 10 presents the serum levels of acylcarnitines and other potential markers for vascular damage. After adjustment for multiple testing, BMI and use of statins, higher levels of C16:1, C18:1, hydroxybutyrylcarnitine (C3-DC(C4-OH)), PC aa C40:6, methionine sulfoxide/methionine (Met-SO/Met, an indicator of systemic oxidative stress), and lower levels of lysoPC a C18:2 were observed in the CAD group compared to the healthy controls.

Associations of arterial stiffness with metabolites

Univariate analysis revealed that cf-PWV was significantly correlated with age ($r=0.63$, $p<0.001$) and eGFR ($\rho=-0.36$, $p=0.012$) for the patients with CAD. After adjusting for multiple comparisons, cf-PWV showed correlations with C14, C16, C16:1, (C2+C3)/C0 (a measure of overall β -oxidation activity), C2/C0 and the carnitine to palmitoyltransferase 1 ratio (the CPT-1 ratio) for the CAD group (see Table 11). For the healthy subjects, cf-PWV correlated significantly only with age ($r=0.51$, $p=0.001$), MAP ($\rho=0.51$, $p=0.001$), AIx@75 ($r=0.51$, $p=0.001$) and glucose levels ($\rho=0.38$, $p=0.017$).

The component scores of factor 3 (acylcarnitines) correlated positively with cf-PWV for the CAD group (see Table 12). This relationship retained significance after adjusting for potential confounders in a multiple regression model ($R^2=0.515$, $p<0.001$, Table 13). For the control group, none of the metabolomic factors identified by PCA were significantly associated with the parameters of arterial stiffness. However, it should be noted that the metabolomic factor that was composed mainly of acylcarnitines showed a borderline significant correlation with cf-PWV (data not shown).

Arterial stiffness-associated metabolites in relation to hemodynamic and biochemical parameters

Table 11 depicts the correlation coefficients of the metabolites related to arterial stiffness for the CAD group. After adjusting for multiple testing the following correlations were observed: factor 3 (acylcarnitines) showed correlations with HDL cholesterol, glucose and adiponectin; C14 correlated with HDL cholesterol

and adiponectin; C16 was correlated with PSBP, CDBP and MAP; (C2+C3)/C0 and C2/C0 showed correlations with adiponectin; the CPT-1 ratio correlated with PSBP, PDBP, CSBP, CDBP, MAP and adiponectin.

Table 9. Hemodynamic and biochemical parameters of the study groups.

Variable	CAD patients (n=52)	Controls (n=40)	p-value
Age (years)	63.2 ± 9.2	60.3 ± 7.1	0.102
BMI (kg/m ²)	27.9 ± 3.5	26.0 ± 3.4	0.013
Height (cm)	176.2 ± 6.4	179.4 ± 6.5	0.021
Peripheral SBP (mmHg)	135.0 ± 14.5	129.5 ± 15.0	0.079
Peripheral DBP (mmHg)	78.5 ± 8.0	77.8 ± 7.3	0.674
Central SBP (mmHg)	122.7 ± 11.1	119.3 ± 13.9	0.205
Central DBP (mmHg)	79.7 ± 7.6	78.6 ± 7.3	0.493
MAP (mmHg)	96.0 (91.6–101.4)	92.5 (89.0–100.0)	0.095
Heart rate (bpm)	62.8 ± 8.3	57.8 ± 7.1	0.003
AIx@75 (%)	18.9 ± 7.2	15.3 ± 8.0	0.029
cf-PWV (m/s)	9.7 ± 2.6	8.2 ± 1.7	0.002
WBC count (x10 ⁹ /L)	6.4 (5.2–7.9)	5.1 (4.7–5.8)	0.002
Total cholesterol (mmol/L)	5.1 ± 1.0	5.5 ± 0.9	0.136
HDL cholesterol (mmol/L)	1.5 ± 0.4	1.6 ± 0.4	0.123
LDL cholesterol (mmol/L)	3.2 (2.8–4.1)	3.6 (3.3–4.2)	0.076
Triglycerides (mmol/L)	1.2 (0.8–1.6)	1.0 (0.7–1.5)	0.264
oxLDL (U/L)	67.2 (53.5–80.7)	71.4 (59.4–90.0)	0.139
Glucose (mmol/L)	5.8 (5.3–6.3)	5.8 (5.4–6.1)	0.81
hsCRP (mg/L)	1.3 (0.7–2.8)	1.2 (0.7–2.1)	0.441
MPO (ng/ml)	58.4 (35.3–110.7)	45.8 (27.7–62.6)	0.023
IL-6 (pg/ml)	1.33 (0.74–1.98)	0.95 (0.64–1.29)	0.03
Adiponectin (ng/ml)	5701 ± 2890	7192 ± 3585	0.043
eGFR (ml/min/1.73 m ²)	86 (71–97)	85 (79–90)	0.953
Current smoking, n (%)	13 (24.5)	5 (12.2)	0.186

The values are expressed as means ± standard deviation, medians (interquartile range), or prevalence (%). AIx@75, augmentation index adjusted to a heart rate of 75 beats per minute; BMI, body mass index; CAD, coronary artery disease; cf-PWV, carotid–femoral pulse wave velocity; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; MAP, mean arterial pressure; MPO, myeloperoxidase; oxLDL, oxidized low-density lipoprotein; SBP, systolic blood pressure; WBC, white blood cell.

Table 10. Acylcarnitines and other potential markers for vascular damage.

Variable	CAD patients (<i>n</i> =52)	Controls (<i>n</i> =40)	<i>p</i> -value ^a
C2	8.23 (6.8–10.8)	7.24 (6.42–8.74)	0.558
C8	0.16 (0.14–0.21)	0.17 (0.14–0.24)	0.393
C10	0.38 (0.32–0.50)	0.38 (0.31–0.52)	0.397
C12	0.13 (0.11–0.16)	0.13 (0.11–0.16)	0.508
C12:1	0.13 (0.11–0.17)	0.13 (0.11–0.17)	0.709
C14	0.03 (0.03–0.04)	0.03 (0.03–0.03)	0.405
C14:1	0.07 (0.05–0.09)	0.06 (0.04–0.08)	0.584
C14:2	0.02 (0.02–0.03)	0.02 (0.02–0.03)	0.596
C16	0.14 (0.11–0.15)	0.12 (0.09–0.12)	0.191
C16:1	0.04 (0.03–0.04)	0.03 (0.02–0.03)	0.046
C16:2	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.887
C18:1	0.15 (0.12–0.17)	0.12 (0.10–0.14)	0.028
C18:2	0.05 (0.04–0.06)	0.04 (0.04–0.05)	0.468
C3-DC(C4-OH)	0.05 (0.04–0.06)	0.04 (0.04–0.05)	0.042
(C2+C3) / C0	0.27 (0.22–0.33)	0.24 (0.21–0.30)	0.754
C2/C0	0.25 (0.21–0.31)	0.23 (0.19–0.28)	0.724
CPT-I ratio	0.005 (0.005–0.006)	0.005 (0.005–0.006)	0.348
lysoPC C18:2	34.6 (28–41.65)	45.3 (40.23–60.25)	0.018
PC aa C40:6	30.1 ± 8.9	23.9 ± 6.6	0.046
Met-SO/Met	0.031 (0.027–0.036)	0.028 (0.024–0.032)	0.022

Concentrations of all metabolites are presented as μM . The values are expressed as means \pm standard deviation or medians (interquartile range). Data were adjusted for multiple testing, body mass index and statin use. Abbreviations: C2, acetylcarnitine; C8, octanoylcarnitine; C10, decanoylcarnitine; C12, dodecanoylcarnitine; C12:1, dodecenoylcarnitine, C14, tetradecanoylcarnitine; C14:1, tetradecenoylcarnitine; C16, hexadecanoylcarnitine; C16:1, hexadecenoylcarnitine; C16:2, hexadeca-dienylcarnitine; C18:1, octadecenoylcarnitine; C3-DC(C4-OH), 3-hydroxybutyrylcarnitine; (C2+C3)/C0, (acetylcarnitine+propionylcarnitine)/carnitine; (C2/C0), acetylcarnitine/carnitine; CAD, coronary artery disease; lysoPC a C18:2, lysophosphatidylcholine acyl C18:2; PC aa C40:6, phosphatidylcholine diacyl C40:6; Met, methionine; Met-SO, methionine sulphoxide. The values that were significantly different between the study groups are shown in bold. ^aBenjamini-Hochberg adjusted *p*-value.

Table 11. Pearson correlation coefficients of the metabolites related to arterial stiffness for the CAD patients.

	<i>Factor 3 (acyl-carnitines)</i>	<i>C14</i>	<i>C16</i>	<i>C16:1</i>	<i>(C2+C3)/C0</i>	<i>C2/C0</i>	<i>CPT-1 ratio</i>
Age	0.36	0.27	0.1	0.31	0.25	0.24	0.21
BMI	-0.03	-0.12	0.13	0.03	-0.07	-0.07	-0.1
PSBP	0.17	0.24	0.36*	0.15	0.28	0.27	0.54**
PDBP	-0.08	0.07	0.32	-0.03	0.09	0.09	0.38*
CSBP	0.2	0.21	0.31	0.19	0.28	0.27	0.53**
CDBP	0.03	0.17	0.41*	0.1	0.17	0.18	0.4*
MAP	0.13	0.21	0.39*	0.1	0.18	0.18	0.46**
cf-PWV	0.49*	0.47*	0.44*	0.5*	0.45*	0.45*	0.47*
Aix@75	-0.1	-0.3	-0.27	-0.17	-0.13	-0.13	-0.07
WBC count	0.29	0.3	0.15	0.12	0.09	0.08	0.00
Total cholesterol	-0.11	0.06	0.23	-0.05	-0.05	-0.05	0.18
HDL cholesterol	0.41*	0.34*	0.1	0.29	0.19	0.19	0.15
LDL cholesterol	-0.19	-0.01	0.21	-0.14	-0.17	-0.18	0.18
Triglycerides	0.01	0.05	0.3	0.08	0.00	-0.01	0.02
oxLDL	-0.00	0.13	0.29	0.12	0.07	0.07	0.24
Glucose	0.38*	0.16	0.09	0.17	0.14	0.14	0.00
hsCRP	-0.32	-0.11	-0.05	-0.25	-0.18	-0.18	0.09
MPO	0.02	-0.13	-0.09	-0.1	0.02	0.03	-0.14
IL-6	0.03	0.15	0.23	0.07	0.18	0.17	0.22
Adiponectin	0.35*	0.44**	0.14	0.24	0.3*	0.3*	0.33*
eGFR	-0.37	-0.2	-0.07	-0.21	-0.28	-0.28	-0.18

Abbreviations: C14, tetradecanoylcarnitine; C16, hexadecanoylcarnitine; C16:1, hexadecenoylcarnitine; (C2+C3)/C0, ratio of short chain acylcarnitines to free carnitine; C2/C0, ratio of acetylcarnitine to free carnitine; BMI, body mass index; CAD, coronary artery disease; CPT-1 ratio, ratio of long chain acylcarnitines to free carnitine; CDBP, central diastolic blood pressure; cf-PWV, carotid-femoral pulse wave velocity; Chol, cholesterol; CSBP, central systolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; MAP, mean arterial pressure; MPO, myeloperoxidase; oxLDL, oxidized low-density lipoprotein; PDBP, peripheral diastolic blood pressure; PSBP, peripheral systolic blood pressure; WBC, white blood cell. Statistically significant correlation coefficients are shown in bold. *Benjamini-Hochberg adjusted *p*-value <0.05, **Benjamini-Hochberg adjusted *p*-value <0.01.

Table 12. Principal component analysis for the coronary artery disease group.

Factor	Description	Components	Eigenvalue	Variance
1	Phosphatidylcholines (PC) / sphingomyelins (SM)	PC aa C42:0, PC ae C32:1, PC ae C34:0, PC ae C34:1, PC ae C34:2, PC ae C34:3, PC ae C36:1, PC ae C36:2, PC ae C36:3, PC ae C36:4, PC ae C38:3, PC ae C38:4, PC ae C38:5, PC ae C38:2, PC ae C40:2, PC ae C40:3, PC ae C40:4, PC ae C40:5, PC ae C40:6, PC ae C42:4, PC ae C42:5, PC ae C44:5, PC ae C44:6, SM (OH) C14:1, SM (OH) C16:1, SM (OH) C22:1, SM (OH) C22:2, SM (OH) C24:1, SM C16:0, Total SM, Total SM-non OH, Total SM-OH	32.64	0.16
2	PCs / lysophosphatidylcholines (lysoPC)	lysoPC a C24:0, lysoPC a C26:0, lysoPC a C26:1, lysoPC a C28:0, lysoPC a C28:1, PC aa C24:0, PC aa C26:0, PC aa C40:2, PC aa C42:6, PC ae C30:0, PC ae C30:1, PC ae C30:2, PC ae C32:2, PC ae C40:1, PC ae C42:0, PC ae C42:1, PC ae C42:3, PC ae C44:3	22.02	0.10
3	Short-, medium-, and long-chain acylcarnitines	C2, C8, C10, C12, C12:1, C14, C14:1, C14:2, C16, C16:1, C16:2, C18:1, C3-DC(C4-OH), (C2+C3)/C0, C2/C0	18.06	0.09
4	Amino acids / short-chain acylcarnitine	Ala, His, Ile, Leu, Phe, Pro, Thr, Trp, Tyr, Val, alpha-AAA, AAA, BCAA, essential AA, glucogenic AA, non-essential AA, total AA, C3	12.52	0.06
5	PCs / lysoPC / polyunsaturated fatty acids (PUFA)	lysoPC a C16:1, PC aa C34:3, PC aa C40:4, PC aa C40:5, PUFA	10.84	0.05

Table 13. Multiple regression model for the CAD patients with cf-PWV as the dependent variable.

Variable	Regression coefficient	Standard error	<i>p</i> -value
Age	0.175	0.034	<0.001
hsCRP	0.511	0.197	0.013
Factor 3 (acylcarnitines)	0.703	0.324	0.036
Statin treatment	-1.192	0.602	0.055

$R^2=0.515$, $p<0.001$. CAD, coronary artery disease; cf-PWV, carotid-femoral pulse wave velocity; hsCRP, high-sensitivity C-reactive protein.

5.4. Serum phosphatidylcholines and lysophosphatidylcholines are inversely related to aortic stiffness, endothelial dysfunction and heart rate in patients with atherosclerosis (Papers III and IV)

Baseline characteristics of the study population

The hemodynamic and biochemical characteristics of the three study groups are summarized in Table 14. There were no significant differences in mean age, PSDP, CDBP, eGFR, ADMA, ADMA/Arg, total cholesterol, LDL cholesterol, glucose, insulin levels or PLT count. The patients with PAD differed statistically significantly from both the CAD and control subjects with respect to CSBP, AIx@75, cf-PWV, Arg, triglycerides, WBC count, hsCRP, IL-6 and to the prevalence of current smoking, antihypertensive therapy and statin use. Differences in the levels of PSBP, MAP, heart rate, HDL cholesterol and in the proportions of antiplatelet therapy only emerged from comparison to the controls. Higher BMI values were observed in the CAD group than in the patients with PAD. The CAD group also had significantly increased cf-PWV, heart rate, WBC count and higher prevalence of antihypertensive, antiplatelet and statin therapy compared to the controls.

Table 15 depicts a selection of glycerophospholipids measured in this study. After adjusting for multiple comparisons, BMI, current smoking status and statin therapy, the PAD patients had significantly lower serum levels of PC aa C28:1, PC aa C30:0, PC aa C32:2, PC ae C30:0 and PC ae C34:2 than the healthy control group (Figure 4). In addition, lower levels of PC aa C32:2 and lysoPC a C18:2 were observed in the patients with CAD compared to the controls (Figure 4).

Relationships of arterial stiffness, endothelial dysfunction biomarkers and resting heart rate with glycerophospholipids

Table 16 depicts the correlation coefficients of lysoPC a C16:0, lysoPC a C17:0, lysoPC a C18:0, lysoPC a C20:4 and PC aa C32:2 for the three study groups. After adjustment for multiple testing, cf-PWV showed negative correlation with serum levels of PC aa C32:2, lysoPC a C16:0 and lysoPC a C18:0 for the PAD patients. LysoPC a C20:4 served as an inverse correlate of cf-PWV for the CAD group, whereas no significant relationships between arterial stiffness and individual lipid species were observed for the control subjects. In multiple regression analysis, however, above mentioned lipids lost their significant relationships to cf-PWV for the both patient groups (data not shown).

Resting heart rate correlated negatively with lysoPC a C17:0, lysoPC a C18:0 and PC aa C32:2 for the PAD patients, but not for the CAD patients or controls (Table 16). After adjusting for potential confounders, lysoPC a C17:0 retained significance as a determinant of heart rate for the PAD group (Table 17).

LysoPC a C20:4, lysoPC a C16:0 and lysoPC a C18:0 were inversely related to ADMA/Arg only for the subjects with PAD. ADMA correlated negatively with lysoPC a C20:4, lysoPC a C18:0 and PC aa C32:2 for the PAD group, whereas lysoPC a C16:0 showed inverse relationship with ADMA for both the PAD and CAD patients (Table 16). Multiple regression analysis revealed, however, that after adjusting for potential confounders, only lysoPC a C20:4 remained a significant determinant of both ADMA/Arg and ADMA for the PAD group, while lysoPC a C18:0 showed the same for the patients with CAD (Table 18). Finally, lysoPC a C20:4 correlated inversely with the angiographic score for the PAD patients (Table 16). However, this relationship lost statistical significance after adjustment for potential confounders (data not shown).

Glycerophospholipids in relation to biochemical parameters

After adjusting for multiple comparisons, the following relationships were found (Table 16): lysoPC a C16:0 showed correlations with total cholesterol, IL-6 and hsCRP for the PAD patients and with total cholesterol and LDL cholesterol for the CAD patients; lysoPC a C17:0 was correlated with IL-6 and hsCRP for the patients with PAD and with IL-6 and insulin for the CAD and control group, respectively; lysoPC a C18:0 showed correlations with total cholesterol, LDL cholesterol, HDL cholesterol, IL-6, hsCRP and insulin for the PAD group, with total cholesterol, LDL cholesterol and IL-6 for the CAD group and with insulin for the healthy controls; lysoPC a C20:4 correlated with IL-6 for the PAD patients and with HDL cholesterol and insulin for the controls; PC aa C32:2 correlated with total cholesterol, LDL cholesterol, and IL-6 only for the PAD group.

Table 14. Baseline characteristics of study participants.

Variable	PAD patients (n=32)	CAD patients (n=52)	Healthy controls (n=40)	p-value
Age (years)	61.7 ± 9.0	63.2 ± 9.2	60.3 ± 7.1	0.27
BMI (kg/m ²)	25.8 ± 3 ^b	27.9 ± 3.5	26 ± 3.4	0.02
Peripheral SBP (mmHg)	142.5 ± 17.7 ^a	135 ± 14.5	129.5 ± 15.0	0.003
Peripheral DBP (mmHg)	79.7 ± 7.5	78.5 ± 8.0	77.8 ± 7.3	0.59
Central SBP (mmHg)	132.7 ± 16.5 ^{a,b}	122.7 ± 11.1	119.3 ± 13.9	<0.001
Central DBP (mmHg)	80.7 ± 7.8	79.7 ± 7.6	78.6 ± 7.3	0.51
MAP (mmHg)	102.5 (95–107.5) ^a	96.0 (91.6–101.4)	92.5 (89.0–100.0)	0.01
Heart rate (bpm)	65.5 (57.5–74.5) ^a	62 (58–68) ^a	57.3 (53–62.5)	0.001
AIx@75 (%)	28 ± 7.4 ^{a,b}	18.9 ± 7.2	15.3 ± 8.0	<0.001
cf-PWV (m/s) ^c	10.5 ± 2.9 ^{a,b}	9.7 ± 2.6 ^a	8.2 ± 1.7	0.007
ADMA (μM)	0.46 (0.42–0.57)	0.43 (0.35–0.52)	0.44 (0.37–0.49)	0.07
Arginine (μM)	122.7 ± 22.7 ^{a,b}	102.4 ± 21	99.4 ± 22.3	<0.001
ADMA/arginine	0.004 (0.003–0.005)	0.004 (0.003–0.005)	0.005 (0.003–0.005)	0.36
Total cholesterol (mmol/L)	5.3 ± 1.1	5.1 ± 1.0	5.5 ± 0.9	0.43
HDL cholesterol (mmol/L)	1.3 ± 0.4 ^a	1.5 ± 0.4	1.6 ± 0.4	0.003
LDL cholesterol (mmol/L)	3.4 (2.7–4.2)	3.2 (2.8–4.1)	3.6 (3.3–4.2)	0.19
Triglycerides (mmol/L)	1.6 (1.4–2.1) ^{a,b}	1.2 (0.8–1.6)	1.0 (0.7–1.5)	0.002
Glucose (mmol/L)	5.5 (5.2–5.9)	5.8 (5.3–6.3)	5.8 (5.4–6.1)	0.07
Insulin (IU/ml)	4.9 (3.1–8.2)	5.8 (4.4–8.8)	4.9 (3.3–7.5)	0.13
WBC count (x10 ⁹ /L)	8.2 (6.3–9.1) ^{a,b}	6.4 (5.2–7.9) ^a	5.1 (4.7–5.8)	<0.001
Platelet count (x10 ⁹ /L)	240 (192–298)	213 (182–247)	210 (186–239)	0.18
hsCRP (mg/L)	3.2 (1.1–6.8) ^{a,b}	1.3 (0.7–2.8)	1.2 (0.7–2.1)	<0.001
Interleukin-6 (pg/ml)	2.81 (1.14–5.57) ^{a,b}	1.33 (0.74–1.98)	0.95 (0.64–1.29)	<0.001
Resistin (ng/ml)	3.5 (2.6–4.4)	3.4 (2.6–4.5)	2.8 (2.3–3.7)	0.18
eGFR (ml/min/1.73 m ²)	89.5 (76–96)	86 (71–97)	85 (79–90)	0.39
Angiographic score (AU)	27.8 ± 7.1	ND	ND	ND
Current smoking, n (%)	25 (78) ^{a,b}	13 (25)	5 (13)	<0.001
Medication, n (%)				
Antihypertensive therapy	19 (59) ^{a,b}	48 (92) ^a	0 (0)	<0.001
Antiplatelet therapy	13 (41) ^a	34 (65) ^a	0 (0)	<0.001
Statin therapy	7 (22) ^{a,b}	28 (54) ^a	0 (0)	<0.001

^a = <0.05 vs control group^b = <0.05 vs CAD group^c = cf-PWV has been adjusted for MAP

The values are presented as means \pm standard deviation, medians (interquartile range), or prevalence (%). ADMA, asymmetric dimethylarginine; AIx@75, augmentation index adjusted to a heart rate of 75 beats per minute; BMI, body mass index; CAD, coronary artery disease; cf-PWV, carotid–femoral pulse wave velocity; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MAP, mean arterial pressure; PAD, peripheral arterial disease; SBP, systolic blood pressure; WBC, white blood cell.

Table 15. Phosphatidylcholine and lysophosphatidylcholine levels among the study groups.

Metabolite	PAD patients (n=32)	CAD patients (n=52)	Healthy controls (n=40)	p-value
lysoPC a C16:0	127 (117–152)	136 (122–156)	148 (128–159)	0.353
lysoPC a C17:0	2.2 (1.7–2.4)	2.4 (1.8–2.9)	2.6 (2.1–3.5)	0.325
lysoPC a C18:0	40.9 (33–46.6)	40.3 (34.4–49.2)	42.2 (37.7–53.5)	0.586
lysoPC a C18:1	25.9 (21.7–35.6)	30 (23.8–34.6)	34.1 (28.2–60.3)	0.325
lysoPC a C18:2	34 (26.2–41.9)	34.6 (28–41.7)^a	45.3 (40.2–60.3)	0.028
lysoPC a C20:3	2.6 (2.2–3.2)	2.4 (2–3.2)	2.9 (2.4–3.7)	0.402
lysoPC a C20:4	8.7 (7.3–10.6)	9.1 (7.4–10.4)	8.6 (7.1–11.2)	0.842
PC aa C28:1	2.8 (2.3–3.1)^a	3 (2.7–3.5)	3.5 (3–4.2)	0.048
PC aa C30:0	3.2 (2.6–3.8)^a	3.3 (2.8–4)	4.3 (3.3–5.5)	0.028
PC aa C32:2	2.1 (1.5–2.8)^a	2.5 (1.9–3.1)^a	3.4 (2.7–5)	0.006
PC aa C34:3	10.1 (7.4–11.4)	9.8 (7.7–12)	13 (10.5–15.4)	0.094
PC aa C36:6	0.69 (0.51–0.98)	0.89 (0.61–1.1)	0.89 (0.7–1.2)	0.353
PC aa C40:5	7.3 (6.8–9.5)	7.2 (6–8.8)	6.5 (5.3–8)	0.404
PC ae C30:0	0.31 (0.23–0.38)^a	0.36 (0.27–0.45)	0.44 (0.35–0.6)	0.028
PC ae C34:2	7.1 (5.7–9)^a	8 (6.7–9.9)	9.6 (8–11.2)	0.048
PC ae C34:3	4.9 (3.7–5.8)	5.5 (4.3–6.5)	6 (5–6.9)	0.325
PC ae C36:3	4.8 (3.8–5.9)	5.5 (4.5–6.5)	6.2 (5–6.8)	0.13
PC ae C36:4	10.6 (9.5–13.1)	13.2 (10–15.7)	12.5 (10.1–14.4)	0.342
PC ae C38:0	1.5 (1.3–2)	1.8 (1.3–2.2)	1.7 (1.5–2.2)	0.483
PC ae C38:3	3.1 (2.7–3.4)	2.9 (2.5–3.4)	3.2 (2.7–3.5)	0.664
PC ae C40:1	1.1 (1–1.5)	1.2 (1.1–1.5)	1.3 (1.1–1.7)	0.325
PC ae C42:1	0.42 (0.36–0.65)	0.42 (0.36–0.62)	0.46 (0.37–0.71)	0.559

^a = <0.05 vs control group

Concentrations of all metabolites are presented as μM . The values are expressed as medians (interquartile range). Abbreviations: a, acyl; aa, diacyl; ae, acyl-alkyl; CAD, coronary artery disease; lysoPC, lysophosphatidylcholine; PAD, peripheral arterial disease. **p*-values were adjusted for body mass index, current smoking status and statin use. Benjamini-Hochberg procedure was used to account for multiple testing. A corrected *p*-value <0.05 was considered to be statistically significant.

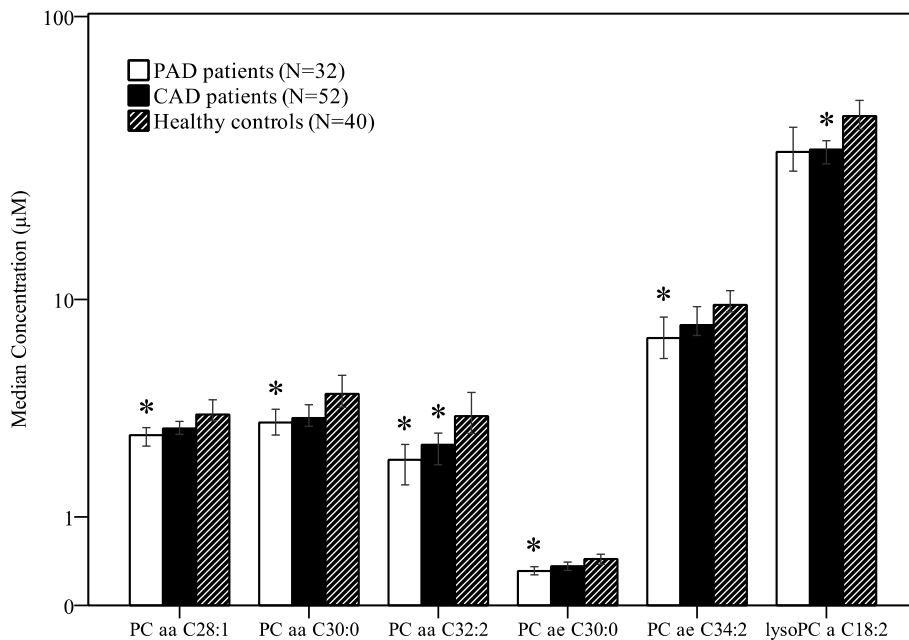


Figure 4. A bar chart showing the median concentrations of selected phosphatidylcholine (PC) and lysophosphatidylcholine (lysoPC) species across the three study groups. **p*-values were adjusted for body mass index, current smoking status and statin use. Benjamini-Hochberg procedure was used to account for multiple testing. A corrected *p*-value <0.05 was considered to be statistically significant. Abbreviations: CAD, coronary artery disease; PAD, peripheral arterial disease.

Table 16. Spearman's rank correlation coefficients between clinical characteristics and selected glycerophospholipids for the three study groups.

	<i>lysoPC a</i> <i>C16:0</i>	<i>lysoPC a</i> <i>C17:0</i>	<i>lysoPC a</i> <i>C18:0</i>	<i>lysoPC a</i> <i>C20:4</i>	<i>PC aa</i> <i>C32:2</i>
<i>PAD patients (n=32)</i>					
cf-PWV	-0.57*	-0.16	-0.45*	-0.28	-0.6**
Heart rate	-0.34	-0.56**	-0.45*	-0.3	-0.47*
Angiographic score	-0.34	-0.05	-0.2	-0.41*	0.01
ADMA	-0.49*	-0.24	-0.44*	-0.5*	-0.42*
ADMA/Arginine	-0.47*	-0.15	-0.38*	-0.44*	-0.21
Total cholesterol	0.54*	0.09	0.58*	0.16	0.66**
LDL cholesterol	0.45	-0.01	0.47*	0.07	0.56**
HDL cholesterol	0.29	0.13	0.47*	0.07	0.32
Interleukin-6	-0.54*	-0.65**	-0.6**	-0.49*	-0.44*
hsCRP	-0.42*	-0.54**	-0.42*	-0.18	-0.38
Insulin	-0.38	-0.22	-0.42*	-0.38	-0.31
<i>CAD patients (n=52)</i>					
cf-PWV	-0.08	-0.04	-0.11	-0.36*	-0.04
Heart rate	-0.07	-0.18	-0.12	-0.26	-0.13
ADMA	-0.32*	-0.04	-0.26	-0.15	0.01
ADMA/Arginine	-0.28	-0.08	-0.22	-0.1	0.03
Total cholesterol	0.56**	0.16	0.55**	0.22	0.37
LDL cholesterol	0.47**	0.11	0.55**	0.24	0.27
HDL cholesterol	0.11	0.12	-0.04	-0.12	0.27
Interleukin-6	-0.14	-0.36*	-0.31*	-0.19	-0.21
hsCRP	-0.09	-0.21	-0.11	0.05	-0.06
Insulin	-0.07	-0.1	-0.06	-0.24	0.17
<i>Healthy controls (n=40)</i>					
cf-PWV	0.18	0.1	0.1	-0.06	0.05
Heart rate	-0.01	-0.27	-0.13	0.05	0.23
ADMA	-0.18	-0.2	-0.2	-0.09	-0.15
ADMA/Arginine	-0.21	-0.18	-0.2	-0.08	-0.13
Total cholesterol	0.25	0.22	0.31	0.27	0.13
LDL cholesterol	0.13	0.13	0.26	0.12	0.1
HDL cholesterol	0.21	0.34	0.29	0.4*	0.1
Interleukin-6	0.02	-0.26	-0.2	0.01	-0.19
hsCRP	-0.19	-0.33	-0.17	0.07	-0.01
Insulin	-0.24	-0.43*	-0.38*	-0.37*	0.09

Abbreviations: a, acyl; aa, diacyl; ADMA, asymmetric dimethylarginine; ae, acyl-alkyl; CAD, coronary artery disease; cf-PWV, carotid-femoral pulse wave velocity; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; lysoPC, lysophosphatidylcholine; PAD, peripheral arterial disease; PC, phosphatidylcholine. Statistically significant correlation coefficients are presented in bold. *Benjamini-Hochberg adjusted *p*-value <0.05, **Benjamini-Hochberg adjusted *p*-value <0.01.

Table 17. Multiple regression model for PAD group with resting heart rate as the dependent variable.

Variable	Regression coefficient	Standard error	<i>p</i> -value
LysoPC a C17:0	-7.962	2.302	0.002
Glucose	9.192	3.110	0.006
Mean arterial pressure	0.309	0.138	0.033

$R^2=0.593$, $p<0.001$. LysoPC, lysophosphatidylcholine; PAD, peripheral arterial disease.

Table 18. Multiple regression analyses for the PAD and CAD patients with ADMA/Arg and ADMA as dependent variables.

Variable	Regression coefficient	Standard error	<i>p</i> -value
PAD patients			
ADMA/Arg ^a			
LysoPC a C20:4	-0.0002	6.7^{-5}	0.006
Mean arterial pressure	4.6^{-5}	1.7^{-5}	0.012
LDL cholesterol	0.0004	0.0002	0.025
ADMA ^b			
LysoPC a C20:4	-0.024	0.009	0.010
LDL cholesterol	-0.034	0.023	0.155
Mean age	0.002	0.003	0.426
CAD patients			
ADMA/Arg ^c			
Platelets	-9.7^{-6}	3.0^{-6}	0.005
Interleukin-6	7.4^{-5}	2.6^{-5}	0.008
LysoPC a C18:0	-4.9^{-5}	2.1^{-5}	0.022
ADMA ^d			
Resistin	0.031	0.012	0.010
LysoPC a C18:0	-0.004	0.002	0.013
LDL cholesterol	0.028	0.016	0.086

^a $R^2=0.388$, $p=0.001$; ^b $R^2=0.300$, $p=0.005$; ^c $R^2=0.273$, $p<0.001$; ^d $R^2=0.162$, $p=0.009$. ADMA, asymmetric dimethylarginine; Arg, arginine; CAD, coronary artery disease; LDL, low-density lipoprotein; lysoPC, lysophosphatidylcholine; PAD, peripheral arterial disease.

6. DISCUSSION

6.1. Relationships between biomarkers of renal damage, arterial stiffness and inflammation in atherosclerotic patients without moderate to severe chronic kidney disease (Papers I and II)

Besides its potential use as a mere reflector of kidney injury (Neiman *et al.* 2011), FBLN-1 serves independent physiological and pathophysiological roles and may directly participate in the development of atherosclerosis and vascular dysfunction (Argraves *et al.* 2009; Cangemi *et al.* 2011; Kostka *et al.* 2001; Laugesen *et al.* 2013). In the present thesis, Paper I demonstrated independent association between serum FBLN-1 concentration and AIX@75 in subjects with PAD. Moreover, significantly higher FBLN-1 and AIX@75 levels were found in these patients compared to the healthy controls. Although we also detected a statistically non-significant increase in circulating levels of FBLN-1 in patients with CAD, no correlation was found between this extracellular protein and AIX@75 for this group. These findings may suggest that the relationship between FBLN-1 and left ventricular late-systolic loading becomes more apparent in distal diffuse atherosclerosis commonly seen in patients with PAD. Yet, it must be noted that the same relationship was recently demonstrated in a group of 210 CAD patients (Hansen and Rasmussen 2015). Thus, the lack of correlation between FBLN-1 and AIX@75 among the CAD patients in our study might have also been due to the relatively small sample size. Previous studies in subjects with type 2 diabetes and CKD showed that FBLN-1 correlated with cf-PWV and unadjusted AIX, respectively, although the latter relationship did not retain statistical significance in multivariate analysis (Laugesen *et al.* 2013; Scholze *et al.* 2013).

A significant correlation between serum FBLN-1 and biochemical markers of inflammation was also observed. Serum resistin emerged as an independent determinant of FBLN-1 concentration in the PAD patients. Increased serum levels of this adipokine have previously been associated with insulin resistance and atherosclerosis (Reilly *et al.* 2005; Syed Ikmal *et al.* 2013). Elevated resistin levels are also associated with CAD and the risk of CV death (Sinan *et al.* 2014). Interestingly, in our study, no significant differences between groups were found in the concentrations of this pro-inflammatory adipokine. These results may imply that the correlation between FBLN-1 and resistin for the PAD group may have appeared due to more extensive vascular injury and increased FBLN-1 levels in these patients compared to the CAD and control groups.

Higher WBC count was also present in both the PAD and CAD patients. High WBC count may reflect a chronic inflammatory state and have been reported to affect vascular function in individuals with low CV risk (Li *et al.* 2013). Leukocytes play an important role in the genesis of atherosclerosis as they represent the predominant cell population involved in inflammation

(Shaykhiev and Bals 2007). Univariate correlation between WBC count and serum FBLN-1 was found in the PAD and CAD patients, and it retained statistical significance in multivariate analysis in the CAD group. This could suggest that the systemic inflammatory state, common in atherosclerosis, has an effect on FBLN-1 serum levels. Whether the same correlation is observed in non-atherosclerotic patients with high WBC count remains to be elucidated.

Increased PLT aggregation and activation have been previously observed in patients with PAD (Robless *et al.* 2003). Furthermore, thrombotic events like MI and stroke are the common causes of morbidity and mortality in these patients (Golomb *et al.* 2006). The potential role of FBLN-1 in hemostasis and thrombosis has been addressed as well. Several studies indicate that FBLN-1 can mediate PLT adhesion by binding fibrinogen, is able to incorporate into fibrin clots and accumulates in human atherosclerotic lesions (Argraves *et al.* 2009). In accordance with the role of FBLN-1 in PLT adhesion, a correlation between FBLN-1 and PLT count in the PAD group was found in the present study. Furthermore, PAI-1, an important protein in fibrinolysis as well as in tissue remodeling, correlated with FBLN-1 in the control group (Ghosh and Vaughan 2012). Although interesting, the exact mechanisms behind these findings cannot be explained within the scope of our study.

In the current thesis, Paper II revealed correlations between aortic stiffness and markers of both renal function and tubular damage in the symptomatic CAD patients. The novel findings that urinary L-FABP and KIM-1 are independently associated with cf-PWV in CAD extend our current knowledge of these proteins and may partially explain their value as biomarkers for CV prognosis in different study populations (Araki *et al.* 2013; Carlsson *et al.* 2013, 2014; Matsumori *et al.* 2012).

L-FABP is involved in the fatty acid uptake, intracellular transport and metabolism (Antonenkov *et al.* 2006; Murphy *et al.* 1996). In the case of kidney damage, the expression of L-FABP in the proximal tubular cells is upregulated (Ferguson *et al.* 2010; Noiri *et al.* 2009; Oyama *et al.* 2005; Yamamoto *et al.* 2007). It has been previously shown that the estimated contribution of serum L-FABP to urinary L-FABP levels is only 3%, hence the L-FABP that is found in urine is produced primarily by the tubular cells (Imai *et al.* 2015; Kamijo *et al.* 2006). Interestingly, the mean urinary levels of this protein were moderately lower in the subjects with CAD compared to the controls in our study, however this difference was not statistically significant. Since L-FABP has been reported as a renoprotective endogenous antioxidant, it can be hypothesized that its lower levels may reflect reduced kidney protection in atherosclerotic patients without moderate to severe CKD (Kanaguchi *et al.* 2011; Matsui *et al.* 2011; Mori *et al.* 2014).

In the presence of increased arterial stiffness, the kidneys are particularly susceptible to the damage resulting from high pulsatile pressure and flow (Mitchell 2008). We observed elevated values of cf-PWV and AIx@75 for the subjects with CAD. Furthermore, besides its independent association with L-FABP and KIM-1, cf-PWV was also correlated with serum creatinine, CysC

and eGFR in these patients, but not in the healthy controls. Although the type of causality could not be determined, these findings support the view that increased arterial stiffness (and thus high pulsatile pressure and flow) contributes to renal dysfunction and propose that urinary L-FABP and KIM-1 may serve as novel biomarkers that reflect this relationship even in the early stages of renal damage. However, it should be noted that this relationship might be bidirectional and could also be affected by some other pathophysiological processes.

We acknowledge that eGFR tends to be less accurate at levels above 60 ml/min per 1.73 m² (Levey *et al.* 2009). However, the Chronic Kidney Disease Epidemiology Collaboration creatinine equation used in the current work performs better than the Modification of Diet in Renal Disease Study formula, especially at higher GFR levels (Levey *et al.* 2009; Stevens *et al.* 2010). When we performed post hoc subgroup analyses based on eGFR levels L-FABP showed a strong and CysC a moderate correlation with cf-PWV for the subgroup of CAD patients with eGFR between 60 and 89 mL/min/1.73 m², whereas none of the measured kidney biomarkers retained as significant correlates of aortic stiffness for the patients with eGFR \geq 90 mL/min/1.73 m². Although these findings are potentially interesting, larger studies with pre-specified subgroup analyses are warranted to explore these possible differences between CAD patients with eGFR \geq 90 mL/min/1.73 m² and those with eGFR between 60 and 89 mL/min/1.73 m².

The relationships between kidney biomarkers and cf-PWV did not change in the control group after dividing subjects into two subgroups based on eGFR (data not shown). The lack of correlations in the healthy subjects might be due to their better preserved vascular function and reduced inflammation compared to the CAD patients. Indeed, decreased serum adiponectin, higher WBC count and elevated IL-6, MPO and resistin levels indicated systemic inflammation in the CAD group when juxtaposed with the healthy controls. Previous findings suggest that elevated resistin may be especially undesirable in the case of reduced adiponectin levels (Machii 2012; Spoto *et al.* 2013). Although the true function of resistin still remains to be established, it seems to play significant regulatory roles in various biological processes including low-grade inflammation and atherosclerosis (Jamaluddin *et al.* 2012).

Urinary KIM-1 showed a borderline significant correlation with cf-PWV for the CAD patients. However, this relationship reached statistical significance after adjusting for potential confounders. KIM-1 functions as a transmembrane phosphatidyl serine receptor that recognizes apoptotic cells and has also been suggested as an early marker for tubular damage (Sabbiseti *et al.* 2014; Torregrosa *et al.* 2015). A recent study in mice demonstrated that by facilitating the phagocytic process, KIM-1 might have a protective anti-inflammatory role in the early stages of AKI (Yang *et al.* 2015). In our work, urinary KIM-1 correlated positively with serum adiponectin and inversely with ferritin for the CAD patients but not for the controls. Although the association between KIM-1 and aortic stiffness in the CAD group was weak, it reveals a novel aspect of this prognostic biomarker and deserves further study.

Several correlations also emerged between serum NGAL and indices of kidney function and inflammation. Positive relationships with WBC count and resistin for both groups are in line with the view that serum NGAL could serve as an inflammatory marker in a range of clinical settings (Gümüs *et al.* 2014; Naudé *et al.* 2013; Smerka *et al.* 2014). This lipocalin is expressed by neutrophils and various epithelial cells and has received attention as an AKI marker because of its early upregulation in the damaged tubular cells (Haase *et al.* 2009; Liebetrau *et al.* 2013). High expression of NGAL in atheromatous plaques and its role in inflammation might explain the previous finding that this biomarker could prove useful in discriminating between unstable and stable CAD patients (Hemdahl *et al.* 2006; Kafkas *et al.* 2012). However, serum NGAL was not related to arterial stiffness in the current study. Further investigations will evidently determine its true role in inflammation and vascular damage.

6.2. Arterial stiffness, hemodynamics and serum acylcarnitines in patients with coronary artery disease (Paper III)

Besides the lipid-related classical CV risk markers, a range of intermediates and products of lipid and amino acid metabolism have emerged as potential indicators of CVD. Association between tyrosine and altered arterial function in patients with PAD has been previously shown by our group (Zagura *et al.* 2015). Moreover, a large prospective study of three population-based cohorts identified phenylalanine – an immediate precursor of tyrosine – and some unsaturated fatty acids as potential biomarkers for CV risk (Würtz *et al.* 2015). These findings indicate that metabolomic studies may help gain a deeper understanding of the molecular mechanisms of CVD. In addition, although different fractions of cholesterol and triglycerides are routinely estimated in clinical practice, they actually cover only a limited part of whole-body lipid metabolism. Therefore, more detailed metabolomic analysis would hopefully lead to the discovery of sensitive and specific novel lipid-related markers for vascular injury and CVD risk.

Paper III demonstrated for the first time, to our knowledge, that the serum acylcarnitine profile is independently associated with aortic stiffness in patients with CAD. While some previous studies have shown the predictive value of acylcarnitines on CV events (Rizza *et al.* 2014; Shah *et al.* 2012), the mechanisms behind this relationship remain poorly understood. Our findings link medium- and long-chain acylcarnitines to cf-PWV and may therefore offer one plausible explanation for the association between these metabolic intermediates and CVD risk.

When the rate of the release of fatty acids from adipose tissue triglycerides overpowers the capacity of β -oxidation, or when the oxidative metabolism of fatty acids in mitochondria is impaired, acylcarnitine levels in the blood will

increase. Therefore, it can be hypothesized that elevated levels of medium- and long-chain acylcarnitines in CAD patients compared to controls may reflect the alterations present at any step in this metabolic pathway (release of fatty acids, their transport into mitochondria, and oxidation in mitochondria). Changes in the activity of the enzymes that regulate long-chain fatty acid transport (e.g. carnitine-palmitoyltransferases 1 (CPT-1) and 2 (CPT-2)) could also occur under hypoxic conditions. Interestingly, we found a positive relationship between the CPT-1 ratio and cf-PWV for the patients with CAD. The CPT-1 ratio reflects the activity of CPT-1 which is a rate-limiting enzyme for β -oxidation of long-chain fatty acids (Michal 1999). In addition, positive correlations between cf-PWV, (C2+C3)/C0 and C2/C0 for the CAD group also suggest a potential link between arterial stiffness and β -oxidation activity for these patients.

Besides its association with arterial stiffness, the PCA-derived factor 3 (acylcarnitines) showed significant correlations with HDL cholesterol, glucose and adiponectin levels for the CAD group. More specifically, C16 correlated with PSBP, CDBP and MAP, and therefore seems to reflect changes both in hemodynamics and aortic stiffness for these patients. Previous studies have demonstrated that C16 might be involved in the pathology of cardiac ischemia. Its accumulation in the myocardial sarcolemma during hypoxia can contribute towards cardiac cell damage and dysfunction (Dhalla *et al.* 1991; McHowat *et al.* 1993; Wu *et al.* 1993). Moreover, high plasma levels of C16 have recently been associated with degree of heart failure (Ueland *et al.* 2013). The same study reported that C16 was also independently associated with all-cause mortality for these patients (Ueland *et al.* 2013). However, little is known about the role of C16:1 in CVD. In the current study, C16:1 levels were higher in the CAD patients compared to the controls and showed the strongest association with arterial stiffness among individual acylcarnitines for the patient group. C16:1 also seems to be a positive marker for adiponectin levels.

The clear association between serum acylcarnitines and arterial stiffness observed for the CAD group is intriguing, yet their true role in vascular injury remains unknown. It is possible that higher serum levels of medium- and long-chain acylcarnitines merely reflect serious cardiac ischemia which is the result of more severe atherosclerosis and increased arterial stiffness. On the other hand, accumulating acylcarnitines can alter insulin signaling and may therefore lead to arterial stiffness through induction of insulin resistance (Schooneman *et al.* 2013; Westerbacka and Yki-Jarvinen 2002). Moreover, insulin resistance itself is accompanied with an increased release of free fatty acids into the bloodstream (Delarue and Magnan 2007). Finally, it can also be hypothesized that acylcarnitines play a direct causal role in vascular injury, however, the true underlying mechanisms of this remain to be established in future experiments.

Several other metabolic variables showed significant positive (C2, C8, C12, C12:1, C14:1, C14:2, C18:1, C18:2, PC aa C42:0, PC aa C42:2) and negative (lysoPC a C20:4) correlations with cf-PWV for the CAD patients before adjusting for multiple comparisons. Apparently, most of these variables were medium- and long-chain acylcarnitines. Interestingly, higher levels of lysoPC a C20:4

were shown to be associated with lower CVD incidence in a previous study by Fernandez and colleagues (Fernandez *et al.* 2013). Although the above relationships between cf-PWV and metabolites lost statistical significance after correcting for multiple testing bias, these findings further support the association between medium- and long-chain acylcarnitines and arterial stiffness reported in the present thesis.

6.3. Arterial stiffness, endothelial dysfunction and resting heart rate in relation to serum phosphatidylcholines and lysophosphatidylcholines in patients with atherosclerosis (Papers III and IV)

Papers III and IV revealed alterations in both PC and lysoPC profiles in the atherosclerotic patients compared to the healthy controls. We also showed that PAD and CAD patients can differ in the way their serum PC and lysoPC levels relate to other biochemical and functional parameters. Thus, despite many similarities, these patient groups cannot be presumed equivalent.

Paper III showed significantly lower concentrations of lysoPC C18:2 in the patients with CAD compared to healthy subjects. This finding is in line with a recent large-scale metabolomic profiling study that demonstrated an inverse relationship between lysoPC C18:2 and CAD (Ganna *et al.* 2014). However, the authors suggested that the association was likely to not be causal. We also observed increased concentrations of PC aa C40:6 in the patients with CAD. The levels of PC aa C40:6 correlated with total cholesterol for both study groups, but a positive relationship with HDL cholesterol was observed only for the CAD group. Interestingly, in a recent prospective study, PC aa C40:6 and lysoPC C18:2 were independently associated with decreased risk of type 2 diabetes (Floegel *et al.* 2013). In our study, PC aa C40:6 showed positive correlation and lysoPC C18:2 showed negative correlation with cf-PWV among the CAD patients, however, these relationships lost statistical significance after adjustment for multiple comparisons. Yet both metabolites might prove their potential as biomarkers of vascular injury in future studies with larger sample size.

Paper IV demonstrated a highly significant correlation between aortic stiffness and PC aa C32:2 for the patients with PAD, but not for the CAD or control group. However, after adjustment for total cholesterol and several other potential confounders, this relationship lost statistical significance (data not shown). It should be noted that before controlling for multiple comparisons (to reduce the risk of type I error), several other PCs (PC aa C34:3, PC aa C40:5, PC ae C36:3, PC ae C36:4, PC ae C38:0, PC ae C38:3 and PC ae C40:1), besides PC aa C32:2, correlated negatively with cf-PWV for the subjects with PAD. Although significantly lower levels of PC aa C32:2 were found in both the PAD and CAD patients compared to the controls, aortic stiffness did not appear to

correlate with this lipid for the subjects with CAD. However, before adjustment for multiple testing, lysoPC a C18:2 and lysoPC a C20:4 correlated negatively and PC aa C40:6 and PC aa 42:2 correlated positively with arterial stiffness for these patients (data not shown).

PCs and lysoPCs serve as reservoirs and transporters of glycerophospholipid components (fatty acids, phosphate, glycerol and choline) (Schmitz and Rueb-saamen 2010). Depletion of these lipid classes might therefore lead to decreased availability of choline which is an important nutrient for humans (Zeisel and Da Costa 2009). Besides being used in membrane phospholipid synthesis, choline is also an essential precursor for the neurotransmitter acetylcholine. Paper IV reported elevated resting heart rate and lower levels of several PCs and lysoPCs in the patient groups compared to the healthy controls. Interestingly, PC and lysoPC molecular species correlated negatively with heart rate for both the patients with PAD and CAD (lysoPC a 17:0, PC aa C30:0, PC aa C32:2, PC aa C36:6 and lysoPC a 18:2, PC ae C34:3, PC ae C40:1, PC ae C42:1, respectively) but not for the controls (data not shown). After adjusting for multiple testing, lysoPC a 17:0 remained a significant correlate of heart rate for the subjects with PAD. This relationship was independent of potential confounding variables. Nevertheless, one can only speculate on the reasons why the patients with lower serum PC and lysoPC levels tended to have increased heart rate in our study. Some of these lipids may have chronotropic effects via direct or indirect modulation of cardiac ion-channels (Kim and Clapham 1989; Wallert *et al.* 1991). A study of a rat heart model showed decreases of heart rate and left ventricular developed pressure after perfusion with exogenous lysoPC (Watanabe and Okada 2003). Thus it seems that there might be a causal rather than a simple correlational relationship between lysoPCs and heart rate. An imbalance between sympathetic and parasympathetic activity plays a prognostic role in patients with CVD (Oberhauser *et al.* 2001) and resting heart rate is an independent predictor of morbidity and mortality in the general population as well as in several types of CVDs (Hjalmarson 1998; Zhang *et al.* 2016). Since decreased heart rate is characteristic of acetylcholine effects, lower PC and lysoPC levels may reflect (or give rise to) decreased parasympathetic drive to the heart. Yet it should be noted that serum choline levels were not measured in this study. It is also plausible that reduced physical performance, inflammation and pain caused by limb or myocardial ischemia lead to increased sympathetic drive and heart rate but lower PC and lysoPC levels in symptomatic patients with atherosclerosis. Evidently, further studies are warranted to clarify the mechanisms behind our findings.

There is a considerable amount of evidence from *ex vivo* studies that lysoPCs induce ED and regulate vascular tone (Kugiyama *et al.* 1999; Murohara *et al.* 1996; Zhang *et al.* 2009). However, less is known about serum levels of individual molecular species of this lipid class in ED. In the present thesis, Paper IV demonstrated relationships between serum lysoPCs and surrogate markers of ED (ADMA and ADMA/Arg). In univariate analysis, after adjusting for multiple testing, lysoPC a C16:0, lysoPC a C18:0, lysoPC a C18:1, lysoPC a

C20:3 and lysoPC a C20:4 remained significant negative correlates of ADMA for the subjects with PAD while lysoPC a C16:0 and lysoPC a 20:3 showed inverse correlation also with ADMA/Arg for these patients. Additionally, lysoPC a C16:0 continued to be a significant negative correlate of ADMA for the CAD patients, whereas no such relationships were found for the healthy controls. In multiple regression analysis, however, lysoPC a C20:4 and lysoPC a C18:0 remained significant determinants of ED for the PAD and CAD patients, respectively. These findings seem paradoxical in the light of previous in vitro studies. However, extrapolating results from ex vivo studies to the whole organism can prove challenging. Plasma concentrations of several lysoPCs have previously been found to be inversely correlated with CRP in obesity (Heimerl *et al.* 2014). Furthermore, lower levels of lysoPCs have also been found in patients with cancer (Taylor *et al.* 2007) and sepsis (Drobnik *et al.* 2003). In our study, the CAD patients had the highest BMI but rarely the lowest levels of individual lysoPC species across the three study groups. This suggests that inflammation (particularly pronounced in PAD patients) might be a more probable cause of the differences in the levels of these lipids. It has been speculated that lower serum levels of lysoPCs in CAD patients might result from their more efficient removal from blood into tissues, either in the form of oxLDL or directly from albumin (Meikle *et al.* 2011). A greater burden of atherosclerosis, and hence worse ED, could therefore coincide with lower serum levels of these lipids. This hypothesis is partially supported by the inverse relationship between lysoPC a C20:4 and angiographic score for the lower extremities observed for the PAD patients in our study. In multivariate analysis, however, this correlation lost its statistical significance (data not shown).

6.4. Possible reasons for observed differences between coronary artery disease patients and peripheral arterial disease patients (Papers I and IV)

Both CAD and PAD patients were included in Papers I and IV. As expected, increased arterial stiffness and inflammation were observed in these atherosclerotic patients compared to the healthy controls (Papers I and IV). In addition, patients with PAD showed worse arterial function than those with CAD. Stiffer arteries may at least partially result from their more pronounced inflammatory status. Indeed, higher WBC count and elevated levels of hsCRP (Papers I and IV) and IL-6 (Paper IV) were observed in the subjects with PAD. It has been previously reported that CAD patients with comorbid PAD have a larger overall burden of atherosclerosis and inflammation, worse endothelial function, increased arterial stiffness and decreased physical activity compared to patients with CAD alone (Grenon *et al.* 2013). Moreover, critical lower extremity ischemia frequently occurs with considerable tissue loss (Aronow 2007), which can alter the levels of circulating metabolites and other biochemical markers.

PAD patients are also less likely to receive antihypertensive and statin therapy (Rice and Lumsden 2006). Accordingly, we found significantly lower rates of antihypertensive medication (50% vs. 95% in Paper I and 59% vs. 92% in Paper IV) and statin use (18% vs. 53% in Paper I and 22% vs. 54% in Paper IV) for the PAD group. Furthermore, cigarette smoking is generally more prevalent in these patients and it has been shown to be a stronger risk factor for PAD than for CAD (Shammas 2007) (72% vs. 21% in Paper I and 78% vs. 25% in Paper IV). Smoking promotes oxidative stress, ED and alters lipid metabolism, among others (Lu and Creager 2004; Shammas 2007).

Finally, lower BMI was found in patients with PAD compared to those with CAD (Papers I and IV). A previous large-scale study in community-living older adults demonstrated that smoking, poor health status, weight loss, and PAD often coexist in older persons, while higher BMI was associated with subsequent PAD in those without these confounding factors, even in older age (Ix *et al.* 2011).

Taken together, all the above mentioned differences in the extent and severity of atherosclerosis as well as in the CVD risk factor prevalence may account for some of the observed differences between these patient groups in the current thesis (Papers I and IV). Despite many similarities, PAD and CAD patients can differ from each other in the way their inflammatory and metabolic profiles relate to other biochemical and functional parameters.

6.5. Limitations

The present study (Papers I–IV) has a number of limitations. Firstly, as the design of our study is cross-sectional, we were unable to establish causality. As such, our data primarily help identify preliminary relationships and generate hypotheses for future research. Secondly, only male participants were recruited and thus our findings cannot be fully extrapolated to women and younger subjects. Thirdly, for ethical reasons, we could not withdraw patients from their medications in order to exclude their potential effects on hemodynamic parameters and levels of biochemical markers. Also, as smokers were included in this study, long-term health consequences of smoking cannot be ruled out. However, in order to minimize some of these confounding effects, all subjects were studied after an overnight fast and abstinence from tobacco, alcohol and caffeine-containing beverages (Papers I–IV).

It should also be noted that various stenotic arterial segments may have led to a considerable underestimation of PWV, particularly in subjects with PAD (Papers I and IV). In addition, the urine albumin-to-creatinine ratio, an early marker of kidney disease, was not determined in Paper II, but should be evaluated in analogous future studies. Finally, we acknowledge that the number of participants in our study was relatively small and larger studies are needed to confirm our results (Papers I–IV).

7. CONCLUSIONS

1. Serum fibulin-1 (FBLN-1) concentrations were significantly increased in non-diabetic peripheral arterial disease patients compared to clinically healthy subjects. Moreover, elevated FBLN-1 levels were independently associated with increased aortic augmentation index for these patients, but not for patients with coronary artery disease or healthy controls. This extracellular protein also seems to be associated with low-grade inflammation in atherosclerosis. Thus, FBLN-1 could serve as a candidate marker for systemic vascular damage.
2. Urinary concentrations of liver-type fatty acid-binding protein (L-FABP) and kidney injury molecule-1 (KIM-1), and serum levels of neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (CysC) did not significantly differ between coronary artery disease patients, with an estimated glomerular filtration rate of above 60 ml/min per 1.73 m², and clinically healthy subjects. Nevertheless, the levels of some of these proteins seem to be at least partially related to inflammation. Most importantly, L-FABP and KIM-1 were independently associated with aortic stiffness for the patient group. Therefore, in subjects with coronary artery disease, measurements of these urinary proteins may help to further explore the interplay between early alterations in renal function and vascular damage.
3. Aortic stiffness was independently associated with serum medium- and long-chain acylcarnitines for patients with coronary artery disease. Serum palmitoylcarnitine and carnitine palmitoyltransferase I also correlated with several hemodynamic parameters in these patients. These findings suggest that in addition to the lipid-related classical cardiovascular disease risk markers, the intermediates of lipid metabolism, such as acylcarnitines, may serve as novel indicators for altered vascular function.
4. The lower serum levels of numerous phosphatidylcholines and lysophosphatidylcholines were related either to increased arterial stiffness, increased resting heart rate or worse endothelial function for peripheral arterial disease patients and coronary artery disease patients but not for healthy controls. Thus, our results may partially explain the previously reported association between decreased circulating concentrations of some of these lipid species and higher cardiovascular risk.
5. Besides many similarities, peripheral arterial disease patients and coronary artery disease patients showed distinct differences in the way their inflammatory and metabolomic profiles were related to other biochemical and functional parameters. Differences in the extent and severity of atherosclerosis as well as in the cardiovascular risk factor prevalence probably account for some of the observed dissimilarities between these patient groups.

8. REFERENCES

- Abdulhannan P, Russell DA, Homer-Vanniasinkam S. Peripheral arterial disease: a literature review. *Br Med Bull* 2012; 104:21–39.
- Abedini S, Meinitzer A, Holme I, Marz W, Weihrauch G, Fellström B, Jardine A, Holdaas H. Asymmetrical dimethylarginine is associated with renal and cardiovascular outcomes and all-cause mortality in renal transplant recipients. *Kidney Int* 2010; 77:44–50.
- Abel ED, Sweeney G. Modulation of the cardiovascular system by leptin. *Biochimie* 2012; 94:2097–2103.
- Abril-Ulloa V, Flores-Mateo G, Solà-Alberich R, Manuel-y-Keenoy B, Arija V. Ferritin levels and risk of metabolic syndrome: meta-analysis of observational studies. *BMC Public Health* 2014; 14:483. doi: 10.1186/1471-2458-14-483.
- Adji A, O'Rourke MF, Namasivayam M. Arterial stiffness, its assessment, prognostic value, and implications for treatment. *Am J Hypertens* 2011; 24:5–17.
- Agner K. Verdoperoxidase: a ferment isolated from leucocytes. *Acta Chem Scand* 1941; 8:1–62.
- Ahmad T, Kelly JP, McGarrah RW, Hellkamp AS, Fiuzat M, Testani JM, Wang TS, Verma A, Samsky MD, Donahue MP, Ilkayeva OR, Bowles DE, Patel CB, Milano CA, Rogers JG, Felker GM, O'Connor CM, Shah SH, Kraus WE. Prognostic implications of long-chain acylcarnitines in heart failure and reversibility with mechanical circulatory support. *J Am Coll Cardiol* 2016; 67:291–299.
- Airaksinen KE, Salmela PI, Linnaluoto MK, Ikäheimo MJ, Ahola K, Ryhänen LJ. Diminished arterial elasticity in diabetes: association with fluorescent advanced glycosylation end products in collagen. *Cardiovasc Res* 1993; 27:942–945.
- Aiyar N, Disa J, Ao Z, Ju H, Nerurkar S, Willette RN, Macphee CH, Johns DG, Douglas SA. Lysophosphatidylcholine induces inflammatory activation of human coronary artery smooth muscle cells. *Mol Cell Biochem* 2007; 295:113–120.
- Akkus MN, Polat G, Yurtdas M, Akcay B, Ercetin N, Cicek D, Doven O, Sucu N. Admission levels of C-reactive protein and plasminogen activator inhibitor-1 in patients with acute myocardial infarction with and without cardiogenic shock or heart failure on admission. *Int Heart J* 2009; 50:33–45.
- Albert MA, Rifai N, Ridker PM. Plasma levels of cystatin-C and mannose binding protein are not associated with risk of developing systemic atherosclerosis. *Vasc Med* 2001; 6:145–149.
- Albrink M, Man E. Serum triglycerides in coronary artery disease. *AMA Arch Intern Med* 1959; 103:4–8.
- Alfadda AA, Sallam RM. Reactive oxygen species in health and disease. *J Biomed Biotechnol*. 2012. Article ID 936486, 14 p. doi:10.1155/2012/936486.
- Anderssohn M, Rosenberg M, Schwedhelm E, Zugck C, Lutz M, Lüneburg N, Frey N, Böger RH. The L-Arginine-asymmetric dimethylarginine ratio is an independent predictor of mortality in dilated cardiomyopathy. *J Card Fail* 2012; 18:904–911.
- Antonenkov VD, Sormunen RT, Ohlmeier S, Amery L, Fransen M, Mannaerts GP, Hiltunen JK. Localization of a portion of the liver isoform of fatty-acid-binding protein (L-FABP) to peroxisomes. *Biochem J* 2006; 394:475–84.
- Araki S, Haneda M, Koya D, Sugaya T, Isshiki K, Kume S, Kashiwagi A, Uzu T, Maegawa H. Predictive effects of urinary liver-type fatty acid-binding protein for deteriorating renal function and incidence of cardiovascular disease in type 2 diabetic patients without advanced nephropathy. *Diabetes Care* 2013; 36:1248–1253.

- Argraves WS, Dickerson K, Burgess WH, Ruoslahti E. Fibulin, a novel protein that interacts with the fibronectin receptor beta-subunit cytoplasmic domain. *Cell* 1989; 58:623–629.
- Argraves WS, Tanaka A, Smith EP, Twal WO, Argraves KM, Fan D, Haudenschild CC. Fibulin-1 and fibrinogen in human atherosclerotic lesions. *Histochem Cell Biol* 2009; 132:559–565.
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; 257:79–83.
- Aronow WS. Peripheral arterial disease in the elderly. *Clin Interv Aging* 2007; 2:645–654.
- Aso Y, Yamamoto R, Wakabayashi S, Uchida T, Takayanagi K, Takebayashi K, Okuno T, Inoue T, Node K, Tobe T, Inukai T, Nakano Y. Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. *Diabetes* 2006; 55:1954–1960.
- Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, O'Rourke MF. Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 1983; 68:50–58.
- Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Münzel T, Simoons ML, Hamm CW. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation* 2003; 108:1440–1445.
- Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B; ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007; 357:2109–2122.
- Basu RK, Wong HR, Krawczeski CD, Wheeler DS, Manning PB, Chawla LS, Devarajan P, Goldstein SL. Combining functional and tubular damage biomarkers improves diagnostic precision for acute kidney injury after cardiac surgery. *J Am Coll Cardiol* 2014; 64:2753–2762.
- Battes LC, Akkerhuis KM, Cheng JM, Garcia-Garcia HM, Oemrawsingh RM, De Boer SPM, Regar E, Van Geuns RJ, Serruys PW, Boersma E, Kardys I. Circulating acute phase proteins in relation to extent and composition of coronary atherosclerosis and cardiovascular outcome: results from the ATHEROREMO-IVUS study. *Int J Cardiol* 2014; 177:847–853.
- Bellien J, Favre J, Iacob M, Gao J, Thuillez C, Richard V, Joannidès R. Arterial stiffness is regulated by nitric oxide and endothelium-derived hyperpolarizing factor during changes in blood flow in humans. *Hypertension* 2010; 55:674–680.
- Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, Bou-touyrie P, Cameron J, Chen CH, Cruickshank JK, Hwang SJ, Lakatta EG, Laurent S, Maldonado J, Mitchell GF, Najjar SS, Newman AB, Ohishi M, Pannier B, Pereira T, Vasan RS, Shokawa T, Sutton-Tyrell K, Verbeke F, Wang KL, Webb DJ, Willum Hansen T, Zoungas S, McEniery CM, Cockcroft JR, Wilkinson IB. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol* 2014; 63:636–646.

- Benetos A, Gautier S, Labat C, Salvi P, Valbusa F, Marino F, Toulza O, Agnoletti D, Zamboni M, Dubail D, Manckoundia P, Rolland Y, Hanon O, Perret-Guillaume C, Lacolley P, Safar ME, Guillemin F. Mortality and cardiovascular events are best predicted by low central/peripheral pulse pressure amplification but not by high blood pressure levels in elderly nursing home subjects: the PARTAGE (Predictive Values of Blood Pressure and Arterial Stiffness in Institutionalized Very Aged Population). *J Am Coll Cardiol* 2012; 60:1503–1511.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 1995; 57:289–300.
- Bjørnbæk C, El-Haschimi K, Frantz JD, Flier JS. The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem* 1999; 274:30059–30065.
- Björkqvist M, Källman J, Fjaertoft G, Xu S, Venge P, Schollin J. Human neutrophil lipocalin: normal levels and use as a marker for invasive infection in the newborn. *Acta Paediatr* 2004; 93:534–539.
- Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med* 2011; 365:2255–2267.
- Böger RH. The pharmacodynamics of L-arginine. *J Nutr* 2007; 137:1650S–1655S.
- Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. *J Immunol* 2005; 174:5789–5795.
- Bolignano D, Donato V, Lacquaniti A, Fazio MR, Bono C, Coppolino G, Buemi M. Neutrophil gelatinase-associated lipocalin (NGAL) in human neoplasias: a new protein enters the scene. *Cancer Lett* 2010; 288:10–16.
- Bonventre JV. Kidney injury molecule-1 (KIM-1): a urinary biomarker and much more. *Nephrol Dial Transpl* 2009; 24:3265–3268.
- Boreham CA, Ferreira I, Twisk JW, Gallagher AM, Savage MJ, Murray LJ. Cardio-respiratory fitness, physical activity, and arterial stiffness: the Northern Ireland Young Hearts Project. *Hypertension* 2004; 44:721–726.
- Böttner A, Kratzsch J, Müller G, Kapellen TM, Blüher S, Keller E, Blüher M, Kiess W. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *J Clin Endocrinol Metab* 2004; 89:4053–4061.
- Bramwell J, Hill A. Velocity of transmission of the pulse-wave and elasticity of arteries. *Lancet* 1922; 199:891–892.
- Brown D, Kinch S, Doyle J. Serum triglycerides in health and in ischemic heart disease. *N Engl J Med* 1965; 273:947–952.
- Brunner L, Nick HP, Cumin F, Chiesi M, Baum HP, Whitebread S, Stricker-Krongrad A, Levens N. Leptin is a physiologically important regulator of food intake. *Int J Obes Relat Metab Disord* 1997; 21:1152–1160.
- Cangemi C, Skov V, Poulsen MK, Funder J, Twal WO, Gall MA, Hjortdal V, Jespersen ML, Kruse TA, Aagard J, Parving HH, Knudsen S, Høilund-Carlsen PF, Rossing P, Henriksen JE, Argraves WS, Rasmussen LM. Fibulin-1 is a marker for arterial extracellular matrix alterations in type 2 diabetes. *Clin Chem* 2011; 57:1556–1565.
- Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM; IMPROVE-IT Investigators. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. *N Engl J Med* 2015; 372:2387–2397.

- Carlsson AC, Larsson A, Helmersson-Karlqvist J, Lind L, Ingelsson E, Larsson TE, Bottai M, Sundström J, Arnlöv J. Urinary kidney injury molecule-1 and the risk of cardiovascular mortality in elderly men. *Clin J Am Soc Nephrol* 2014; 9:1393–1401.
- Carlsson AC, Larsson A, Helmersson-Karlqvist J, Lind L, Ingelsson E, Larsson TE, Sundström J, Arnlöv J. Urinary kidney injury molecule 1 and incidence of heart failure in elderly men. *Eur J Heart Fail* 2013; 15:441–446.
- Casas JP, Shah T, Cooper J, Hawe E, McMahon AD, Gaffney D, Packard CJ, O'Reilly DS, Juhan-Vague I, Yudkin JS, Tremoli E, Margaglione M, Di Minno G, Hamsten A, Kooistra T, Stephens JW, Hurel SJ, Livingstone S, Colhoun HM, Miller GJ, Bautista LE, Meade T, Sattar N, Humphries SE, Hingorani AD. Insight into the nature of the CRP-coronary event association using Mendelian randomization. *Int J Epidemiol* 2006; 35:922–931.
- Caselli C, D'Amico A, Cabiati M, Prescimone T, Del Ry S, Giannessi D. Back to the heart: the protective role of adiponectin. *Pharmacol Res* 2014; 82:9–20.
- Castell JV, Gomez-Lechon MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, Heinrich PC. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 1989; 242:237–239.
- Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA* 1986; 256:2835–2838.
- Cecelja M, Chowienczyk P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: a systematic review. *Hypertension* 2009; 54:1328–1336.
- Cecelja M, Chowienczyk P. Role of arterial stiffness in cardiovascular disease. *JRSM Cardiovasc Dis* 2012; 1:11.
- Charlton J, Portilla D, Okusa M. A basic science view of acute kidney injury biomarkers. *Nephrol Dial Transpl* 2014; 29:1301–1311.
- Chen L, Liang B, Froese DE, Liu S, Wong JT, Tran K, Hatch M, Mymin D, Kroeger EA, Man RYK, Choy PC. Oxidative modification of low density lipoprotein in normal and hyperlipidemic patients: effect of lysophosphatidylcholine composition on vascular relaxation. *J Lipid Res* 1997; 38:546–553.
- Chen SC, Kochan JP, Campfield LA, Burn P, Smeyne RJ. Splice variants of the OB receptor gene are differentially expressed in brain and peripheral tissues of mice. *J Recept Signal Transduct Res* 1999; 19:245–266.
- Chen Y, Budd RC, Kelm RJ, Sobel BE, Schneider DJ. Augmentation of proliferation of vascular smooth muscle cells by plasminogen activator inhibitor type 1. *Arter Thromb Vasc Biol* 2006; 26:1777–1783.
- Cho Y, Lee SE, Lee HC, Hur J, Lee S, Youn SW, Lee J, Lee HJ, Lee TK, Park J, Hwang SJ, Kwon YW, Cho HJ, Oh BH, Park YB, Kim HS. Adipokine resistin is a key player to modulate monocytes, endothelial cells, and smooth muscle cells, leading to progression of atherosclerosis in rabbit carotid artery. *J Am Coll Cardiol* 2010; 57:99–109.
- Chue C, Townend J, Steeds R, Ferro C. Arterial stiffness in chronic kidney disease: causes and consequences. *Heart* 2010; 96:817–823.
- Cooper RA. Influence of increased membrane cholesterol on membrane fluidity and cell function in human red blood cells. *J Supramol Struct* 1978; 8:413–430.
- Correia ML, Morgan DA, Sivitz WI, Mark AL, Haynes WG. Leptin acts in the central nervous system to produce dose-dependent changes in arterial pressure. *Hypertension* 2001; 37:936–942.

- Correia MLG, Haynes WG. A role for plasminogen activator inhibitor-1 in obesity: from pie to PAI? *Arter Thromb Vasc Biol* 2006; 26:2183–2185.
- Covic A, Sirtopol D. Pulse wave velocity ratio: the new ‘gold standard’ for measuring arterial stiffness. *Hypertension* 2015; 65:289–290.
- Cowland JB, Sorensen OE, Sehested M, Borregaard N. Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alpha. *J Immunol* 2003; 171:6630–6639.
- Crandall DL, Quinet EM, El Ayachi S, Hreha AL, Leik CE, Savio DA, Juhan-Vague I, Alessi MC. Modulation of adipose tissue development by pharmacological inhibition of PAI-1. *Arter Thromb Vasc Biol* 2006; 26:2209–2215.
- Criqui MH, Aboyans V. Epidemiology of peripheral artery disease. *Circ Res* 2015; 116:1509–1526.
- Croset M, Brossard N, Polette A, Lagarde M. Characterization of plasma unsaturated lysophosphatidylcholines in human and rat. *Biochem J* 2000; 345 Pt 1:61–67.
- Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006; 52:601–623.
- Dart AM, Gatzka CD, Cameron JD, Kingwell BA, Liang YL, Berry KL, Reid CM, Jennings GL. Large artery stiffness is not related to plasma cholesterol in older subjects with hypertension. *Arter Thromb Vasc Biol* 2004; 24:962–968.
- Daugherty A, Dunn JL, Rateri DL, Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J Clin Invest* 1994; 94:437–444.
- Davies JE, Baksi J, Francis DP, Hadjiloizou N, Whinnett ZI, Manisty CH, Aguado-Sierra J, Foale RA, Malik IS, Tyberg JV, Parker KH, Mayet J, Hughes AD. The arterial reservoir pressure increases with aging and is the major determinant of the aortic augmentation index. *Am J Physiol Heart Circ Physiol* 2010; 298:H580–H586.
- De Haro J, Acin F, Lopez-Quintana A, Medina FJ, Martinez-Aguilar E, Florez A, March JR. Direct association between C-reactive protein serum levels and endothelial dysfunction in patients with claudication. *Eur J Vasc Endovasc Surg* 2008; 35:480–486.
- Delarue J, Magnan C. Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care* 2007; 10:142–148.
- Delaš I, Popović M, Petrović T, Delaš F, Ivanković D. Changes in the Fatty Acid Composition of Brain and Liver Phospholipids from Rats Fed Fat-Free Diet. *FTB* 2008; 46:278–285.
- DePalma RG, Hayes VW, Chow BK, Shamayeva G, May PE, Zacharski LR. Ferritin levels, inflammatory biomarkers, and mortality in peripheral arterial disease: a substudy of the Iron (Fe) and Atherosclerosis Study (FeAST) Trial. *J Vasc Surg* 2010; 51:1498–1503.
- Devaraj S, Xu DY, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation* 2003; 107:398–404.
- Dhalla NS, Kolár F, Shah KR, Ferrari R. Effects of some L-carnitine derivatives on heart membrane ATPases. *Cardiovasc Drugs Ther* 1991; 5:25–30.
- Dharmidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; 40:221–226.
- Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray K, Thompson A, Wood A, Lewington S, Sattar N, Packard C, Collins R, Thompson S, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009; 302:1993–2000.

- Do R, Willer CJ, Schmidt EM, Sengupta S, Gao C, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang HY, Demirkan A, Den Hertog HM, Donnelly LA, Ehret GB, Esko T, Feitosa MF, Ferreira T, Fischer K, Fontanillas P, Fraser RM, Freitag DF, Gurdasani D, Heikkilä K, Hyppönen E, Isaacs A, Jackson AU, Johansson A, Johnson T, Kaakinen M, Kettunen J, Kleber ME, Li X, Luan J, Lyytikäinen LP, Magnusson PK, Mangino M, Mihailov E, Montasser ME, Müller-Nurasyid M, Nolte IM, O'Connell JR, Palmer CD, Perola M, Petersen AK, Sanna S, Saxena R, Service SK, Shah S, Shungin D, Sidore C, Song C, Strawbridge RJ, Surakka I, Tanaka T, Teslovich TM, Thorleifsson G, Van den Herik EG, Voight BF, Volcik KA, Waite LL, Wong A, Wu Y, Zhang W, Absher D, Asiki G, Barroso I, Been LF, Bolton JL, Bonnycastle LL, Brambilla P, Burnett MS, Cesana G, Dimitriou M, Doney AS, Döring A, Elliott P, Epstein SE, Eyjolfsson GI, Gigante B, Goodarzi MO, Grallert H, Gravito ML, Groves CJ, Hallmans G, Hartikainen AL, Hayward C, Hernandez D, Hicks AA, Holm H, Hung YJ, Illig T, Jones MR, Kaleebu P, Kastelein JJ, Khaw KT, Kim E, Klopp N, Komulainen P, Kumari M, Langenberg C, Lehtimäki T, Lin SY, Lindström J, Loos RJ, Mach F, McArdle WL, Meisinger C, Mitchell BD, Müller G, Nagaraja R, Narisu N, Nieminen TV, Nsubuga RN, Olafsson I, Ong KK, Palotie A, Papamarkou T, Pomilla C, Pouta A, Rader DJ, Reilly MP, Ridker PM, Rivadeneira F, Rudan I, Ruukonen A, Samani N, Scharnagl H, Seeley J, Silander K, Stančáková A, Stirrups K, Swift AJ, Tiret L, Uitterlinden AG, van Pelt LJ, Vedantam S, Wainwright N, Wijmenga C, Wild SH, Willemssen G, Wilsgaard T, Wilson JF, Young EH, Zhao JH, Adair LS, Arveiler D, Assimes TL, Bandinelli S, Bennett F, Bochud M, Boehm BO, Boomsma DI, Borecki IB, Bornstein SR, Bovet P, Burnier M, Campbell H, Chakravarti A, Chambers JC, Chen YD, Collins FS, Cooper RS, Danesh J, Dedoussis G, de Faire U, Feranil AB, Ferrières J, Ferrucci L, Freimer NB, Gieger C, Groop LC, Gudnason V, Gyllensten U, Hamsten A, Harris TB, Hingorani A, Hirschhorn JN, Hofman A, Hovingh GK, Hsiung CA, Humphries SE, Hunt SC, Hveem K, Iribarren C, Järvelin MR, Jula A, Kähönen M, Kaprio J, Kesäniemi A, Kivimäki M, Kooner JS, Koudstaal PJ, Krauss RM, Kuh D, Kuusisto J, Kyvik KO, Laakso M, Lakka TA, Lind L, Lindgren CM, Martin NG, März W, McCarthy MI, McKenzie CA, Meneton P, Metspalu A, Moilanen L, Morris AD, Munroe PB, Njølstad I, Pedersen NL, Power C, Pramstaller PP, Price JF, Psaty BM, Quertermous T, Rauramaa R, Saleheen D, Salomaa V, Sanghera DK, Saramies J, Schwarz PE, Sheu WH, Shuldiner AR, Siegbahn A, Spector TD, Stefansson K, Strachan DP, Tayo BO, Tremoli E, Tuomilehto J, Uusitupa M, van Duijn CM, Vollenweider P, Wallentin L, Wareham NJ, Whitfield JB, Wolfenbittel BH, Altshuler D, Ordovas JM, Boerwinkle E, Palmer CN, Thorsteinsdottir U, Chasman DI, Rotter JI, Franks PW, Ripatti S, Cupples LA, Sandhu MS, Rich SS, Boehnke M, Deloukas P, Mohlke KL, Ingelsson E, Abecasis GR, Daly MJ, Neale BM, Kathiresan S. Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet* 2013; 45:1345–1352.
- Drobnik W, Liebisch G, Audebert FX, Frohlich D, Gluck T, Vogel P, Rothe G, Schmitz G. Plasma ceramide and lysophosphatidylcholine inversely correlate with mortality in sepsis patients. *J Lipid Res* 2003; 44:754–761.
- Du Clos TW. Function of C-reactive protein. *Ann Med* 2000; 32:274–278.
- Duivenvoorden R, Holleboom AG, Van Den Bogaard B, Nederveen AJ, De Groot E, Hutten BA, Schimmel AW, Hovingh GK, Kastelein JJP, Kuivenhoven JA, Stroes

- ESG. Cholesterol acyltransferase gene mutations have accelerated atherogenesis as assessed by carotid 3.0-T magnetic resonance imaging: carriers of lecithin. *J Am Coll Cardiol* 2011; 58:2481–2487.
- Dunn OJ. Multiple comparisons using rank sums. *Technometrics* 1964; 6:241–252.
- Eisen A, Hoshen M, Balicer RD, Reges O, Rabi Y, Leibowitz M, Iakobishvili Z, Hasdai D. Estimated glomerular filtration rate within the normal or mildly impaired range and incident cardiovascular disease. *Am J Med* 2015; 128:1015–1022.
- Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, Castro L, Lusis AJ, Nauseef WM, White CR, Freeman BA. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 2002; 296:2391–2394.
- Eitzman DT, Westrick RJ, Nabel EG, Ginsburg D. Plasminogen activator inhibitor-1 and vitronectin promote vascular thrombosis in mice. *Blood* 2000; 95:577–580.
- Ellervik C, Marott JL, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG. Total and cause-specific mortality by moderately and markedly increased ferritin concentrations: general population study and metaanalysis. *Clin Chem* 2014; 60:1419–1428.
- Engelmann B, Schönthier UM, Richter WO, Duhm J. Changes of membrane phospholipid composition of human erythrocytes in hyperlipidemias. II. Increases in distinct molecular species of phosphatidylethanolamine and phosphatidylcholine containing arachidonic acid. *Biochim Biophys Acta* 1992; 1165:38–44.
- Esteve E, Castro A, López-Bermejo A, Vendrell J, Ricart W, Fernández-Real JM. Serum interleukin-6 correlates with endothelial dysfunction in healthy men independently of insulin sensitivity. *Diabetes Care* 2007; 30:939–945.
- Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005; 115:911–920.
- Fasshauer M, Blüher M. Adipokines in health and disease. *Trends Pharmacol Sci* 2015; 36:461–470.
- Fay WP, Garg N, Sunkar M. Vascular functions of the plasminogen activation system. *Arter Thromb Vasc Biol* 2007; 27:1231–1237.
- Fazio S, Linton MF. High-density lipoprotein therapeutics and cardiovascular prevention. *J Clin Lipidol* 2010; 4:411–419.
- Ferguson MA, Vaidya VS, Waikar SS, Collings FB, Sunderland KE, Gioules CJ, Bonventre JV. Urinary liver-type fatty acid-binding protein predicts adverse outcomes in acute kidney injury. *Kidney Int* 2010; 77:708–714.
- Fernandez C, Sandin M, Sampaio JL, Almgren P, Narkiewicz K, Hoffmann M, Hedner T, Wahlstrand B, Simons K, Shevchenko A, James P, Melander O. Plasma lipid composition and risk of developing cardiovascular disease. *PLoS One* 2013; 8(8):e71846. doi: 10.1371/journal.pone.0071846.
- Fischer S, Hanefeld M, Haffner SM, Fusch C, Schwanebeck U, Kohler C, Fucker K, Julius U. Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass. *Acta Diabetol* 2002; 39:105–110.
- Fiseha T. Urinary biomarkers for early diabetic nephropathy in type 2 diabetic patients. *Biomark Res* 2015; 3:16. doi: 10.1186/s40364-015-0042-3.
- Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, Joost HG, Fritsche A, Häring HU, De Angelis MH, Peters A, Roden M, Prehn C, Wang-Sattler R, Illig T, Schulze MB, Adamski J, Boeing H, Pischon T. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes* 2013; 62:639–648.

- Förstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, Kleinert H. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. *Hypertension* 1994; 23:1121–1131.
- Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J* 2012; 33:829–837.
- Fortier C, Mac-Way F, Desmeules S, Marquis K, De Serres SA, Lebel M, Boutouyrie P, Agharazii M. Aortic-brachial stiffness mismatch and mortality in dialysis population. *Hypertension* 2015; 65:378–384.
- Frankel DS, Vasan RS, D’Agostino RB, Benjamin EJ, Levy D, Wang TJ, Meigs JB. Resistin, adiponectin, and risk of heart failure: the Framingham offspring study. *J Am Coll Cardiol* 2009; 53:754–762.
- Freitas Lima LC, Braga V de A, do Socorro de França Silva M, Cruz J de C, Sousa Santos SH, de Oliveira Monteiro MM, Balarini C de M. Adipokines, diabetes and atherosclerosis: an inflammatory association. *Front Physiol* 2015; 6:304. doi: 10.3389/fphys.2015.00304.
- Frostegård J, Wu R, Lemne C, Thulin T, Witztum JL, de Faire U. Circulating oxidized low-density lipoprotein is increased in hypertension. *Clin Sci* 2003; 105:615–620.
- Fu X, Kassim SY, Parks WC, Heinecke JW. Hypochlorous acid oxygenates the cysteine switch domain of pro-matrilysin (MMP-7): a mechanism for matrix metalloproteinase activation and atherosclerotic plaque rupture by myeloperoxidase. *J Biol Chem* 2001; 276:41279–41287.
- Fulcher J, O’Connell R, Voysey M, Emberson J, Blackwell L, Mihaylova B, Simes J, Collins R, Kirby A, Colhoun H, Braunwald E, La Rosa J, Pedersen TR, Tonkin A, Davis B, Sleight P, Franzosi MG, Baigent C, Keech A; Cholesterol Treatment Trialists' (CTT) Collaboration. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials. *Lancet* 2015; 385:1397–1405.
- Furuki K, Adachi H, Enomoto M, Otsuka M, Fukami A, Kumagae S, Matsuoka H, Nanjo Y, Kakuma T, Imaizumi T. Plasma level of asymmetric dimethylarginine (ADMA) as a predictor of carotid intima-media thickness progression: six-year prospective study using carotid ultrasonography. *Hypertens Res* 2008; 31:1185–1189.
- Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res* 2002; 90:251–262.
- Ganna A, Salihovic S, Sundström J, Broeckling CD, Hedman ÅK, Magnusson PKE, Pedersen NL, Larsson A, Siegbahn A, Zilmer M, Prenti J, Ärnlöv J, Lind L, Fall T, Ingelsson E. Large-scale metabolomic profiling identifies novel biomarkers for incident coronary heart disease. *PLoS Genet* 2014; 10(12):e1004801. doi: 10.1371/journal.pgen.1004801.
- Ghantous CM, Azrak Z, Hanache S, Abou-Kheir W, Zeidan A. Differential role of leptin and adiponectin in cardiovascular system. *Int J Endocrinol* 2015; 2015:534320. doi: 10.1155/2015/534320.
- Ghosh AK, Vaughan DE. PAI-1 in tissue fibrosis. *J Cell Physiol* 2012; 227:493–507.
- Golomb BA, Dang TT, Criqui MH. Peripheral arterial disease: morbidity and mortality implications. *Circulation* 2006; 114:688–699.
- Grenon SM, Vittinghoff E, Owens CD, Conte MS, Whooley M, Cohen BE. Peripheral artery disease and risk of cardiovascular events in patients with coronary artery disease: insights from the Heart and Soul Study. *Vasc Med* 2013; 18:176–84.
- Griffin JD, Lichtenstein AH. Dietary cholesterol and plasma lipoprotein profiles: randomized-controlled trials. *Curr Nutr Rep* 2013; 2:274–282.

- Grubb A, Björk J, Nyman U, Pollak J, Bengzon J, Ostner G, Lindström V. Cystatin C, a marker for successful aging and glomerular filtration rate, is not influenced by inflammation. *Scand J Clin Lab Invest* 2011; 71:145–149.
- Grubb A, Löfberg H. Human gamma-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis. *Proc Natl Acad Sci U S A* 1982; 79:3024–3027.
- Gümüş A, Ozkaya S, Ozyurt S, Cinarka H, Kirbas A, Sahin U, Ece F. A novel biomarker in the diagnosis of parapneumonic effusion: neutrophil gelatinase-associated lipocalin. *Multidiscip Respir Med* 2014; 9(1):49. doi: 10.1186/2049-6958-9-49.
- Ha H, Oh EY, Lee HB. The role of plasminogen activator inhibitor 1 in renal and cardiovascular diseases. *Nat Rev Nephrol* 2009; 5:203–211.
- Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A, Bagshaw SM, Bogle R, Changchun C, Constantin JM, Cruz D, Dragun D, Frei U, Goldstein SL, Koyner J, Krawczeski CD, Lima EQ, Ling W, Makris K, Malyszko J, Murray P, Nickolas TL, Puntmann V, Ronco C, Wagener G, Wheeler DS, Xin C, Zappitelli M, Zhaohui N. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009; 54:1012–1024.
- Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 1984; 219:1–14.
- Han WK, Wagener G, Zhu Y, Wang S, Lee HT. Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. *Clin J Am Soc Nephrol* 2009; 4:873–882.
- Hansen ML, Rasmussen LM. Associations between plasma fibulin-1, pulse wave velocity and diabetes in patients with coronary heart disease. *J Diabetes Complicat* 2015; 29:362–366.
- Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352:1685–1695.
- Hanukoglu I. Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. *J Steroid Biochem Mol Biol* 1992; 43:779–804.
- Hao G, Li W, Guo R, Yang JG, Wang Y, Tian Y, Liu MY, Peng YG, Wang ZW. Serum total adiponectin level and the risk of cardiovascular disease in general population: a meta-analysis of 17 prospective studies. *Atherosclerosis* 2013; 228:29–35.
- Hara S, Shike T, Takasu N, Mizui T. Lysophosphatidylcholine promotes cholesterol efflux from mouse macrophage foam cells. *Arter Thromb Vasc Biol* 1997; 17:1258–1266.
- Hartman J, Frishman WH. Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy. *Cardiol Rev* 2014; 22:147–151.
- Hattori Y, Nakano Y, Hattori S, Tomizawa A, Inukai K, Kasai K. High molecular weight adiponectin activates AMPK and suppresses cytokine-induced NF-kappaB activation in vascular endothelial cells. *FEBS Lett* 2008; 582:1719–1724.
- Hayward CS, Kelly RP. Gender-related differences in the central arterial pressure waveform. *J Am Coll Cardiol* 1997; 30:1863–1871.
- Heimerl S, Fischer M, Baessler A, Liebisch G, Sigruener A, Wallner S, Schmitz G. Alterations of plasma lysophosphatidylcholine species in obesity and weight loss. *PLoS One* 2014; 9(10):e111348. doi: 10.1371/journal.pone.0111348.

- Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, Thorén P, Hansson GK. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. *Arter Thromb Vasc Biol* 2006; 26:136–142.
- Henriksen T, Mahoney EM, Steinberg D. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptors for acetylated low density lipoproteins. *Proc Natl Acad Sci U S A* 1981; 78:6499–6503.
- Heslop CL, Frohlich JJ, Hill JS. Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography. *J Am Coll Cardiol* 2010; 55:1102–1109.
- Hingorani AD, Casas JP. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet* 2012; 379:1214–1224.
- Hirano T, Taga T, Matsuda T, Hibi M, Suematsu S, Tang B, Murakami M, Kishimoto T. Interleukin 6 and its receptor in the immune response and hematopoiesis. *Int J Cell Cloning* 1990; 8 Suppl 1:155–166.
- Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S, Nakajima K, Koyama K, Iwamatsu A. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature* 1986; 324:73–76.
- Hjalmarson A. Significance of reduction in heart rate in cardiovascular disease. *Clin Cardiol* 1998; 21:II3–II7.
- Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 1998; 98:1487–1494.
- Hope SA, Tay DB, Meredith IT, Cameron JD. Comparison of generalized and gender-specific transfer functions for the derivation of aortic waveforms. *Am J Physiol Heart Circ Physiol*. 2002; 283:H1150-H1156.
- Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, Pyörälä K. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. *Arch Intern Med* 2004; 164:1066–1076.
- Huang X, Yang Z. Resistin's, obesity and insulin resistance: the continuing disconnect between rodents and humans. *J Endocrinol Invest* 2015; 39:607–615.
- Ichimura T, Asselton EJ, Humphreys BD, Gunaratnam L, Duffield JS, Bonventre JV. Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. *J Clin Invest* 2008; 118:1657–1668.
- Ichimura T, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL, Sanicola M. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *J Biol Chem* 1998; 273:4135–4142.
- Ichimura T, Hung CC, Yang SA, Stevens JL, Bonventre JV. Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury. *Am J Physiol Ren Physiol* 2004; 286:F552–F563.
- Ikonomidis I, Michalakeas CA, Parissis J, Paraskevaidis I, Ntai K, Papadakis I, Anastasiou-Nana M, Lekakis J. Inflammatory markers in coronary artery disease. *Biofactors* 2012; 38:320–328.
- Imai N, Yasuda T, Kamijo-Ikemori A, Shibagaki Y, Kimura K. Distinct roles of urinary liver-type fatty acid-binding protein in non-diabetic patients with anemia. *PLoS One* 2015; 10(5):e0126990. doi: 10.1371/journal.pone.0126990.

- Imatoh T, Miyazaki M, Momose Y, Tanihara S, Une H. Adiponectin levels associated with the development of hypertension: a prospective study. *Hypertens Res* 2008; 31:229–233.
- Isabelle M, Simonet S, Ragonnet C, Sansilvestri-Morel P, Clavreul N, Vayssettes-Courchay C, Verbeuren TJ. Chronic reduction of nitric oxide level in adult spontaneously hypertensive rats induces aortic stiffness similar to old spontaneously hypertensive rats. *J Vasc Res* 2012; 49:309–318.
- Ix JH, Biggs ML, Kizer JR, Mukamal KJ, Djousse L, Ziemann SJ, de Boer IH, Nelson TL, Newman AB, Criqui MH, Siscovick DS. Association of body mass index with peripheral arterial disease in older adults: the Cardiovascular Health Study. *Am J Epidemiol* 2011; 174:1036–1043.
- Jamaluddin MS, Weakley SM, Yao Q, Chen C. Resistin: functional roles and therapeutic considerations for cardiovascular disease. *Br J Pharmacol* 2012; 165:622–632.
- Janner JH, Godtfredsen NS, Ladelund S, Vestbo J, Prescott E. High aortic augmentation index predicts mortality and cardiovascular events in men from a general population, but not in women. *Eur J Prev Cardiol* 2013; 20:1005–1012.
- Jatoi N, Jerrard-Dunne P, Feely J, Mahmud A. Impact of smoking and smoking cessation on arterial stiffness and aortic wave reflection in hypertension. *Hypertension* 2007; 49:981–985.
- Jones NL, Reagan JW, Willingham MC. The pathogenesis of foam cell formation: modified LDL stimulates uptake of co-incubated LDL via macropinocytosis. *Arter Thromb Vasc Biol* 2000; 20:773–781.
- Jørgensen AB, Frikke-Schmidt R, West AS, Grande P, Nordestgaard BG, Tybjaerg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J* 2013; 34:1826–1833.
- Kafkas N, Demponeras C, Zoubouloglou F, Spanou L, Babalis D, Makris K. Serum levels of gelatinase associated lipocalin as indicator of the inflammatory status in coronary artery disease. *Int J Inflamm* 2012; 2012:189797. doi: 10.1155/2012/189797.
- Kakimoto Y, Akazawa S. Isolation and identification of N-G,N-G- and N-G,N⁷-G-dimethyl-arginine, N-epsilon-mono-, di-, and trimethyllysine, and glucosylgalactosyl- and galactosyl-delta-hydroxylysine from human urine. *J Biol Chem* 1970; 245:5751–5758.
- Kalantar-Zadeh K, Don BR, Rodriguez RA, Humphreys MH. Serum ferritin is a marker of morbidity and mortality in hemodialysis patients. *Am J Kidney Dis* 2001; 37:564–572.
- Kals J, Kampus P, Kals M, Teesalu R, Zilmer K, Pulges A, Zilmer M. Arterial elasticity is associated with endothelial vasodilatory function and asymmetric dimethylarginine level in healthy subjects. *Scand J Clin Lab Invest* 2007; 67:536–544.
- Kamijo A, Sugaya T, Hikawa A, Yamanouchi M, Hirata Y, Ishimitsu T, Numabe A, Takagi M, Hayakawa H, Tabei F, Sugimoto T, Mise N, Omata M, Kimura K. Urinary liver-type fatty acid binding protein as a useful biomarker in chronic kidney disease. *Mol Cell Biochem* 2006; 284:175–182.
- Kampus P, Kals J, Ristimäe T, Muda P, Ulst K, Zilmer K, Salonen RM, Tuomainen TP, Teesalu R, Zilmer M. Augmentation index and carotid intima-media thickness are differently related to age, C-reactive protein and oxidized low-density lipoprotein. *J Hypertens* 2007; 25:819–825.

- Kampus P, Muda P, Kals J, Ristimäe T, Fischer K, Teesalu R, Zilmer M. The relationship between inflammation and arterial stiffness in patients with essential hypertension. *Int J Cardiol* 2006; 112:46–51.
- Kanaguchi Y, Suzuki Y, Osaki K, Sugaya T, Horikoshi S, Tomino Y. Protective effects of L-type fatty acid-binding protein (L-FABP) in proximal tubular cells against glomerular injury in anti-GBM antibody-mediated glomerulonephritis. *Nephrol Dial Transpl* 2011; 26:3465–3473.
- Katagiri M, Takahashi M, Doi K, Myojo M, Kiyosue A, Ando J, Hirata Y, Komuro I. Serum neutrophil gelatinase-associated lipocalin concentration reflects severity of coronary artery disease in patients without heart failure and chronic kidney disease. *Heart Vessels* 2015. doi:10.1007/s00380-015-0776-8.
- Kell DB, Pretorius E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics* 2014; 6:748–773.
- Kim D, Clapham DE. Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. *Science* 1989; 244:1174–1176.
- Kivimäki M, Lawlor DA, Eklund C, Smith GD, Hurme M, Lehtimäki T, Viikari JSA, Raitakari OT. Mendelian randomization suggests no causal association between C-reactive protein and carotid intima-media thickness in the young Finns study. *Arter Thromb Vasc Biol* 2007; 27:978–979.
- Kjeldsen L, Johnsen AH, Sengeløv H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *J Biol Chem* 1993; 268:10425–10432.
- Knight EL, Verhave JC, Spiegelman D, Hillege HL, De Zeeuw D, Curhan GC, De Jong PE. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 2004; 65:1416–1421.
- Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res* 2004; 94:27–31.
- Koenig W, Khuseynova N, Baumert J, Meisinger C, Löwel H. Serum concentrations of adiponectin and risk of type 2 diabetes mellitus and coronary heart disease in apparently healthy middle-aged men: results from the 18-year follow-up of a large cohort from southern Germany. *J Am Coll Cardiol* 2006; 48:1369–1377.
- Koenig W, Twardella D, Brenner H, Rothenbacher D. Plasma concentrations of cystatin C in patients with coronary heart disease and risk for secondary cardiovascular events: more than simply a marker of glomerular filtration rate. *Clin Chem* 2005; 51:321–327.
- Kohn JC, Lampi MC, Reinhart-King CA. Age-related vascular stiffening: causes and consequences. *Front Genet* 2015; 6:1–17.
- Konijn AM, Hershko C. Ferritin synthesis in inflammation. I. Pathogenesis of impaired iron release. *Br J Haematol* 1977; 37:7–16.
- Konstantinides S, Schäfer K, Loskutoff DJ. Do PAI-1 and vitronectin promote or inhibit neointima formation? The exact role of the fibrinolytic system in vascular remodeling remains uncertain. *Arter Thromb Vasc Biol* 2002; 22:1943–1945.
- Kostka G, Giltay R, Bloch W, Addicks K, Timpl R, Fässler R, Chu ML. Perinatal lethality and endothelial cell abnormalities in several vessel compartments of fibulin-1-deficient mice. *Mol Cell Biol* 2001; 21:7025–7034.
- Krzyzanowska K, Mittermayer F, Wolzt M, Schernthaner G. Asymmetric dimethylarginine predicts cardiovascular events in patients with type 2 diabetes. *Diabetes Care* 2007; 30:1834–1839.

- Kugiyama K, Sugiyama S, Ogata N, Oka H, Doi H, Ota Y, Yasue H. Burst production of superoxide anion in human endothelial cells by lysophosphatidylcholine. *Atherosclerosis* 1999; 143:201–204.
- Kume N, Gimbrone Jr M. Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. *J Clin Invest* 1994; 93:907–911.
- Kutter D, Devaquet P, Vanderstocken G, Paulus JM, Marchal V, Gothot A. Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit? *Acta Haematol* 2000; 104:10–15.
- Landin K, Stigendal L, Eriksson E, Krotkiewski M, Risberg B, Tengborn L, Smith U. Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. *Metabolism* 1990; 39:1044–1048.
- Lane T, Wassef N, Poole S, Mistry Y, Lachmann HJ, Gillmore JD, Hawkins PN, Pepys MB. Infusion of pharmaceutical-grade natural human C-reactive protein is not proinflammatory in healthy adult human volunteers. *Circ Res* 2014; 114:672–676.
- Laugesen E, Høyem P, Christiansen JS, Knudsen ST, Hansen KW, Argraves WS, Hansen TK, Poulsen PL, Rasmussen LM. Plasma levels of the arterial wall protein fibulin-1 are associated with carotid-femoral pulse wave velocity: a cross-sectional study. *Cardiovasc Diabetol* 2013; 12:107.
- Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; 37:1236–1241.
- Laurent S, Cockcroft JR, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson IB, Struijker-Boudier H. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; 27:2588–2605.
- Lee S, Lee HC, Kwon YW, Lee SE, Cho Y, Kim J, Lee S, Kim JY, Lee J, Yang HM, Mook-Jung I, Nam KY, Chung J, Lazar MA, Kim HS. Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes. *Cell Metab* 2014; 19:484–497.
- Lehrer RI, Hanifin J, Cline MJ. Defective bactericidal activity in myeloperoxidase-deficient human neutrophils. *Nature* 1969; 223:78–79.
- Leiper J, Nandi M. The therapeutic potential of targeting endogenous inhibitors of nitric oxide synthesis. *Nat Rev Drug Discov* 2011; 10:277–291.
- Lekawanvijit S, Kompa AR, Zhang Y, Wang BH, Kelly DJ, Krum H. Myocardial infarction impairs renal function, induces renal interstitial fibrosis, and increases renal KIM-1 expression: implications for cardiorenal syndrome. *Am J Physiol Heart Circ Physiol* 2012; 302:H1884–H1893.
- Leng GC, Lee AJ, Fowkes FG, Lowe GD, Housley E. The relationship between cigarette smoking and cardiovascular risk factors in peripheral arterial disease compared with ischaemic heart disease. The Edinburgh Artery Study. *Eur Heart J* 1995; 16:1542–1548.
- Leung-Tack J, Tavera C, Gensac MC, Martinez J, Colle A. Modulation of phagocytosis-associated respiratory burst by human cystatin C: role of the N-terminal tetrapeptide Lys-Pro-Pro-Arg. *Exp Cell Res* 1990; 188:16–22.
- Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro 3rd AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150:604–612.

- Li J, Flammer AJ, Reriani MK, Matsuo Y, Gulati R, Friedman PA, Thomas RJ, Sandhu NP, Lerman LO, Lerman A. High leukocyte count is associated with peripheral vascular dysfunction in individuals with low cardiovascular risk. *Circ J* 2013; 77:780–785.
- Libby P, Ridker PM, Hansson G. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* 2009; 54:2129–2138.
- Liebetrau C, Dörr O, Baumgarten H, Gaede L, Szardien S, Blumenstein J, Rolf A, Möllmann H, Hamm C, Walther T, Nef H, Weber M. Neutrophil gelatinase-associated lipocalin (NGAL) for the early detection of cardiac surgery associated acute kidney injury. *Scand J Clin Lab Invest* 2013; 73:392–9.
- Lindberg S, Jensen JS, Bjerre M, Pedersen SH, Frystyk J, Flyvbjerg A, Galatius S, Jeppesen J, Mogelvang R. Adiponectin, type 2 diabetes and cardiovascular risk. *Eur J Prev Cardiol* 2015; 22:276–283.
- Lindberg S, Jensen JS, Mogelvang R, Pedersen SH, Galatius S, Flyvbjerg A, Magnusson NE. Plasma neutrophil gelatinase-associated lipocalin in the general population: association with inflammation and prognosis. *Arter Thromb Vasc Biol* 2014; 34:2135–2142.
- Lindberg S, Pedersen SH, Mogelvang R, Jensen JS, Flyvbjerg A, Galatius S, Magnusson NE. Prognostic utility of neutrophil gelatinase-associated lipocalin in predicting mortality and cardiovascular events in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention. *J Am Coll Cardiol* 2012; 60:339–345.
- Liu FD, Shen XL, Zhao R, Tao XX, Wang S, Zhou JJ, Zheng B, Zhang QT, Yao Q, Zhao Y, Zhang X, Wang XM, Liu HQ, Shu L, Liu JR. Pulse pressure as an independent predictor of stroke: a systematic review and a meta-analysis. *Clin Res Cardiol* 2016; 105:677–686.
- London GM, Blacher J, Pannier B, Guérin AP, Marchais SJ, Safar ME. Arterial wave reflections and survival in end-stage renal failure. *Hypertension* 2001; 38:434–438.
- Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; 394:897–901.
- Lu JT, Creager MA. The relationship of cigarette smoking to peripheral arterial disease. *Rev Cardiovasc Med* 2004; 5:189–193.
- Lu Y, Vaarhorst A, Merry AHH, Dollé MET, Hovenier R, Imholz S, Schouten LJ, Heijmans BT, Müller M, Slagboom PE, Van den Brandt PA, Gorgels APM, Boer JMA, Feskens EJM. Markers of endogenous desaturase activity and risk of coronary heart disease in the CAREMA cohort study. *PLoS One* 2012; 7(7):e41681. doi: 10.1371/journal.pone.0041681.
- Luo J, Wang LP, Hu HF, Zhang L, Li YL, Ai LM, Mu HY, Kun-Wang. Cystatin C and cardiovascular or all-cause mortality risk in the general population: a meta-analysis. *Clin Chim Acta* 2015; 450:39–45.
- Luttun A, Lupu F, Storkebaum E, Hoylaerts MF, Moons L, Crawley J, Bono F, Poole AR, Tipping P, Herbert JM, Collen D, Carmeliet P. Lack of plasminogen activator inhibitor-1 promotes growth and abnormal matrix remodeling of advanced atherosclerotic plaques in apolipoprotein E-deficient mice. *Arter Thromb Vasc Biol* 2002; 22:499–505.
- Machii N. Resistin exacerbates insulin resistance under the condition of low adiponectin in 3T3-L1 adipocytes. *J Diabetes Metab* 2012; 03:1–6.

- Maeda Y, Suzuki A, Ishii J, Sekiguchi-Ueda S, Shibata M, Yoshino Y, Asano S, Hayakawa N, Nakamura K, Akiyama Y, Kitagawa F, Sakuishi T, Fujita T, Hashimoto S, Ozaki Y, Itoh M. Level of urinary liver-type fatty acid-binding protein is associated with cardiac markers and electrocardiographic abnormalities in type-2 diabetes with chronic kidney disease stage G1 and G2. *Heart Vessels* 2014; 30:362–368.
- Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension* 2005; 46:1118–1122.
- Maisel AS, Mueller C, Fitzgerald R, Brikhan R, Hiestand BC, Iqbal N, Clopton P, Van Veldhuisen DJ. Prognostic utility of plasma neutrophil gelatinase-associated lipocalin in patients with acute heart failure: the NGAL Evaluation Along with B-type Natriuretic Peptide in acutely decompensated heart failure (GALLANT) trial. *Eur J Heart Fail* 2011; 13:846–851.
- Maiwall R, Kumar S, Chaudhary AK, Maras J, Wani Z, Kumar C, Rastogi A, Bihari C, Vashisht C, Sarin SK. Serum ferritin predicts early mortality in patients with decompensated cirrhosis. *J Hepatol* 2014; 61:43–50.
- Mäki-Petäjä KM, Hall FC, Booth AD, Wallace SML, Yasmin, Bearcroft PWP, Harish S, Furlong A, McEnery CM, Brown J, Wilkinson IB. Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy. *Circulation* 2006; 114:1185–1192.
- Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F, Redon J, Dominiczak A, Narkiewicz K, Nilsson PM, Burnier M, Viigimaa M, Ambrosioni E, Caulfield M, Coca A, Olsen MH, Schmieder RE, Tsioufis C, van de Borne P, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S, Clement DL, Coca A, Gillebert TC, Tendera M, Rosei EA, Ambrosioni E, Anker SD, Bauersachs J, Hitij JB, Caulfield M, De Buyzere M, De Geest S, Derumeaux GA, Erdine S, Farsang C, Funck-Brentano C, Gerc V, Germano G, Gielen S, Haller H, Hoes AW, Jordan J, Kahan T, Komajda M, Lovic D, Mahrholdt H, Olsen MH, Ostergren J, Parati G, Perk J, Polonia J, Popescu BA, Reiner Z, Rydén L, Sirenko Y, Stanton A, Struijker-Boudier H, Tsioufis C, van de Borne P, Vlachopoulos C, Volpe M, Wood DA. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013; 34:2159–2219.
- Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord* 2002;26:1407–1433.
- Mark AL, Correia MLG, Rahmouni K, Haynes WG. Selective leptin resistance: a new concept in leptin physiology with cardiovascular implications. *J Hypertens* 2002; 20:1245–1250.
- Mark AL. Selective leptin resistance revisited. *Am J Physiol Regul Integr Comp Physiol* 2013; 305:R566–R581.
- Matsui K, Kamijo-Ikemori A, Sugaya T, Yasuda T, Kimura K. Renal liver-type fatty acid binding protein (L-FABP) attenuates acute kidney injury in aristolochic acid nephrotoxicity. *Am J Pathol* 2011; 178:1021–1032.

- Matsumori R, Shimada K, Kiyonagi T, Hiki M, Fukao K, Hirose K, Ohsaka H, Miyazaki T, Kume A, Yamada A, Takagi A, Ohmura H, Miyauchi K, Daida H. Clinical significance of the measurements of urinary liver-type fatty acid binding protein levels in patients with acute coronary syndrome. *J Cardiol* 2012; 60:168–173.
- Matsumoto T, Kobayashi T, Kamata K. Mechanisms underlying lysophosphatidylcholine-induced potentiation of vascular contractions in the Otsuka Long-Evans Tokushima Fatty (OLETF) rat aorta. *Br J Pharmacol* 2006; 149:931–941.
- Mattace-Raso F, Hofman A, Verwoert G, Wittemana J, Wilkinson IB, Cockcroft JR, McEniery CM, Yasmin, Laurent S, Boutouyrie P, Bozec E, Hansen T, Torp-Pedersen C, Ibsen H, Jeppesen J, Vermeersch S, Rietzschel E, De Buyzere M, Gillebert T, Van Bortel L, Segers P, Vlachopoulos C, Aznaouridis C, Stefanadis C, Benetos A, Labat C, Lacolley P, Stehouwer C, Nijpels G, Dekker J, Stehouwer C, Ferreira I, Twisk J, Czernichow S, Galan P, Herberg S, Pannier B, Guérin A, London G, Cruickshank J, Anderson S, Paini A, Agabiti Rosei E, Muiesan M, Salvetti M, Filipovsky J, Seidlerova J, Dolejsova M. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: establishing normal and reference values. *Eur Heart J* 2010; 31:2338–2350.
- Mattace-Raso FUS, Van Der Cammen TJM, Hofman A, Van Popele NM, Bos ML, Schalekamp MADH, Asmar R, Reneman RS, Hoeks APG, Breteler MMB, Witteman JCM. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation* 2006; 113:657–663.
- McEniery CM, Spratt M, Munnery M, Yarnell J, Lowe G, Rumley A, Gallacher J, Ben-Shlomo Y, Cockcroft JR, Wilkinson IB. An analysis of prospective risk factors for aortic stiffness in men: 20-year follow-up from the Caerphilly prospective study. *Hypertension* 2010a; 56:36–43.
- McEniery CM, Yasmin, Maki-Petaja KM, McDonnell BJ, Munnery M, Hickson SS, Franklin SS, Cockcroft JR, Wilkinson IB. The impact of cardiovascular risk factors on aortic stiffness and wave reflections depends on age: the Anglo-Cardiff Collaborative Trial (ACCT III). *Hypertension* 2010b; 56:591–597.
- McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, Wilkinson IB. Central blood pressure: current evidence and clinical importance. *Eur Heart J* 2014; 35:1719–1725.
- McEniery CM, Wallace S, MacKenzie IS, McDonnell B, Newby DE, Cockcroft JR, Wilkinson IB. Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertension* 2006;48:602–608.
- McHowat J, Yamada KA, Saffitz JE, Corr PB. Subcellular distribution of endogenous long chain acylcarnitines during hypoxia in adult canine myocytes. *Cardiovasc Res* 1993; 27:1237–1243.
- McLeod LL, Sevanian A. Lipid peroxidation and modification of lipid composition in an endothelial cell model of ischemia and reperfusion. *Free Radic Biol Med* 1997; 23:680–694.
- McTernan C, McTernan P, Harte A, Levick P, Barnett A, Kumar S. Resistin, central obesity, and type 2 diabetes. *Lancet* 2002; 359:46–47.
- Medić B, Rovčanin B, Basta Jovanović G, Radojević-Škodrić S, Prostran M. Kidney injury molecule-1 and cardiovascular diseases: from basic science to clinical practice. *Biomed Res Int* 2015; 2015:854070. doi: 10.1155/2015/854070.
- Meikle PJ, Wong G, Tsorotes D, Barlow CK, Weir JM, Christopher MJ, MacIntosh GL, Goudey B, Stern L, Kowalczyk A, Haviv I, White AJ, Dart AM, Duffy SJ, Jennings

- GL, Kingwell BA. Plasma lipidomic analysis of stable and unstable coronary artery disease. *Arter Thromb Vasc Biol* 2011; 31:2723–2732.
- Meisinger C, Baumert J, Khuseynova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation* 2005; 112:651–657.
- Menzaghi C, Bacci S, Salvemini L, Mendonca C, Palladino G, Fontana A, De Bonis C, Marucci A, Goheen E, Prudente S, Morini E, Rizza S, Kanagaki A, Fini G, Mangiacotti D, Federici M, De Cosmo S, Pellegrini F, Doria A, Trischitta V. Serum resistin, cardiovascular disease and all-cause mortality in patients with type 2 diabetes. *PLoS One* 2013; 8(6):e64729. doi: 10.1371/journal.pone.0064729.
- Meuwese MC, Stroes ES, Hazen SL, Van Miert JN, Kuivenhoven JA, Schaub RG, Wareham NJ, Luben R, Kastelein JJ, Khaw KT, Boekholdt SM. Serum myeloperoxidase levels are associated with the future risk of coronary artery disease in apparently healthy individuals: the EPIC-Norfolk Prospective Population Study. *J Am Coll Cardiol* 2007; 50:159–165.
- Michal G. *Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology*. Heidelberg: Spektrum Akademischer Verlag; 1999.
- Millasseau SC, Stewart AD, Patel SJ, Redwood SR, Chowienczyk PJ. Evaluation of carotid-femoral pulse wave velocity: influence of timing algorithm and heart rate. *Hypertension* 2005; 45:222–226.
- Miosge N, Götz W, Sasaki T, Chu ML, Timpl R, Herken R. The extracellular matrix proteins fibulin-1 and fibulin-2 in the early human embryo. *Histochem J* 1996; 28:109–116.
- Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J, Devarajan P. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol* 2003; 14:2534–2543.
- Mitchell GF, Hwang SJ, Vasani RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* 2010; 121:505–511.
- Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasani RS, Levy D. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women. *Hypertension* 2004; 43:1239–1245.
- Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *J Appl Physiol* 2008; 105:1652–1660.
- Mocatta TJ, Pilbrow AP, Cameron VA, Senthilmohan R, Frampton CM, Richards AM, Winterbourn CC. Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction. *J Am Coll Cardiol* 2007; 49:1993–2000.
- Mori Y, Sato N, Kobayashi Y, Ochiai R. Low levels of urinary liver-type fatty acid-binding protein may indicate a lack of kidney protection during aortic arch surgery requiring hypothermic circulatory arrest. *J Clin Anesth* 2014; 26:118–124.
- Mou S, Wang Q, Li J, Shi B, Ni Z. Urinary excretion of liver-type fatty acid-binding protein as a marker of progressive kidney function deterioration in patients with chronic glomerulonephritis. *Clin Chim Acta* 2012; 413:187–191.
- Murohara T, Scalia R, Lefer AM. Lysophosphatidylcholine promotes P-selectin expression in platelets and endothelial cells. Possible involvement of protein kinase C activation and its inhibition by nitric oxide donors. *Circ Res* 1996; 78:780–789.

- Murphy EJ, Prows DR, Jefferson JR, Schroeder F. Liver fatty acid-binding protein expression in transfected fibroblasts stimulates fatty acid uptake and metabolism. *Biochim Biophys Acta* 1996; 1301:191–198.
- Muse ED, Feldman DI, Blaha MJ, Dardari ZA, Blumenthal RS, Budoff MJ, Nasir K, Criqui MH, Cushman M, McClelland RL, Allison MA. The association of resistin with cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis* 2015; 239:101–108.
- Mynard JP, Penny DJ, Davidson MR, Smolich JJ. The reservoir-wave paradigm introduces error into arterial wave analysis: a computer modelling and in-vivo study. *J Hypertens* 2012; 30:734–743.
- Nachman RL, Hajjar KA, Silverstein RL, Dinarello CA. Interleukin 1 induces endothelial cell synthesis of plasminogen activator inhibitor. *J Exp Med* 1986; 163:1595–1600.
- Nasioudis D, Witkin SS. Neutrophil gelatinase-associated lipocalin and innate immune responses to bacterial infections. *Med Microbiol Immunol* 2015; 205:471–479.
- Naudé PJW, Eisel ULM, Comijs HC, Groenewold NA, De Deyn PP, Bosker FJ, Luiten PGM, den Boer JA, Oude Voshaar RC. Neutrophil gelatinase-associated lipocalin: a novel inflammatory marker associated with late-life depression. *J Psychosom Res* 2013; 75:444–450.
- Ndrepepa G, Braun S, Mehilli J, Von Beckerath N, Schömig A, Kastrati A. Myeloperoxidase level in patients with stable coronary artery disease and acute coronary syndromes. *Eur J Clin Invest* 2008; 38:90–96.
- Neely J, Morgan H. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annu Rev Physiol* 1974; 36:413–459.
- Neiman M, Hedberg JJ, Dönnies PR, Schuppe-Koistinen I, Hanschke S, Schindler R, Uhlén M, Schwenk JM, Nilsson P. Plasma profiling reveals human fibulin-1 as candidate marker for renal impairment. *J Proteome Res* 2011; 10:4925–4934.
- Nicholls SJ, Hazen SL. Myeloperoxidase and cardiovascular disease. *Arter Thromb Vasc Biol* 2005; 25:1102–1111.
- Nichols WW, O'Rourke MF. McDonald's Blood Flow in Arteries. Theoretical, Experimental and Clinical Principles. 4th edition. Oxford University Press; 1998.
- Nijveldt RJ, Teerlink T, Van Der Hoven B, Siroen MPC, Kuik DJ, Rauwerda JA, Van Leeuwen PAM. Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality. *Clin Nutr* 2003; 22:23–30.
- Njajou OT, Kanaya AM, Holvoet P, Connelly S, Strotmeyer ES, Harris TB, Cummings SR, Hsueh WC. Association between oxidized LDL, obesity and type 2 diabetes in a population-based cohort, the health, aging and body composition study. *Diabetes Metab Res Rev* 2009; 25:733–739.
- Nkuipou-Kenfack E, Durantou F, Gayraud N, Argilés À, Lundin U, Weinberger KM, Dakna M, Delles C, Mullen W, Husi H, Klein J, Koeck T, Zürgbig P, Mischak H. Assessment of metabolomic and proteomic biomarkers in detection and prognosis of progression of renal function in chronic kidney disease. *PLoS One* 2014; 9(5):e96955. doi: 10.1371/journal.pone.0096955.
- Noiri E, Doi K, Negishi K, Tanaka T, Hamasaki Y, Fujita T, Portilla D, Sugaya T. Urinary fatty acid-binding protein 1: an early predictive biomarker of kidney injury. *Am J Physiol Ren Physiol* 2009; 296:F669–F679.

- Notsu Y, Yano S, Shibata H, Nagai A, Nabika T. Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: Shimane CoHRE study. *Atherosclerosis* 2015; 239:61–66.
- Numaguchi K, Egashira K, Takemoto M, Kadokami T, Shimokawa H, Sueishi K, Takeshita A. Chronic inhibition of nitric oxide synthesis causes coronary microvascular remodeling in rats. *Hypertension* 1995; 26:957–962.
- Nylander M, Kroese A, Strandén E, Morken B, Sandbaek G, Lindahl AK, Arnesen H, Seljeflot I. Markers of vascular inflammation are associated with the extent of atherosclerosis assessed as angiographic score and treadmill walking distances in patients with peripheral arterial occlusive disease. *Vasc Med* 2006; 11:21–28.
- O'Rourke MF, Pauca A, Jiang XJ. Pulse wave analysis. *Br J Clin Pharmacol* 2001; 51:507–522.
- Oberhauser V, Schwertfeger E, Rutz T, Beyersdorf F, Rump LC. Acetylcholine release in human heart atrium: influence of muscarinic autoreceptors, diabetes, and age. *Circulation* 2001; 103:1638–1643.
- Ockner RK, Manning JA, Poppenhausen RB, Ho WK. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. *Science* 1972; 177:56–58.
- Odobasic D, Kitching AR, Holdsworth SR. Neutrophil-mediated regulation of innate and adaptive immunity: the role of myeloperoxidase. *J Immunol Res* 2016; 2016:2349817. doi: 10.1155/2016/2349817.
- Odobasic D, Kitching AR, Yang Y, O'Sullivan KM, Muljadi RCM, Edgton KL, Tan DSY, Summers SA, Morand EF, Holdsworth SR. Neutrophil myeloperoxidase regulates T-cell-driven tissue inflammation in mice by inhibiting dendritic cell function. *Blood* 2013; 121:4195–4204.
- Ogawa T, Kimoto M, Sasaoka K. Purification and properties of a new enzyme, NG,NG-dimethylarginine dimethylaminohydrolase, from rat kidney. *J Biol Chem* 1989; 264:10205–10209.
- Ohashi K, Shibata R, Murohara T, Ouchi N. Role of anti-inflammatory adipokines in obesity-related diseases. *Trends Endocrinol Metab* 2014; 25:348–355.
- Okui H, Hamasaki S, Ishida S, Kataoka T, Orihara K, Fukudome T, Ogawa M, Oketani N, Saihara K, Shinsato T, Shirasawa T, Mizoguchi E, Kubozono T, Ichiki H, Ninomiya Y, Matsushita T, Nakasaki M, Tei C. Adiponectin is a better predictor of endothelial function of the coronary artery than HOMA-R, body mass index, immunoreactive insulin, or triglycerides. *Int J Cardiol* 2008; 126:53–61.
- Ouchi N, Ohashi K, Shibata R, Murohara T. Adipocytokines and obesity-linked disorders. *Nagoya J Med Sci* 2012; 74:19–30.
- Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogihara T, Matsuzawa Y. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension* 2003; 42:231–234.
- Oyama Y, Takeda T, Hama H, Tanuma A, Iino N, Sato K, Kaseda R, Ma M, Yamamoto T, Fujii H, Kazama JJ, Odani S, Terada Y, Mizuta K, Gejyo F, Saito A. Evidence for megalin-mediated proximal tubular uptake of L-FABP, a carrier of potentially nephrotoxic molecules. *Lab Invest* 2005; 85:522–531.
- Pääkkö T, Ukkola O, Ikäheimo M, Kesäniemi YA. Plasma adiponectin levels are associated with left ventricular hypertrophy in a random sample of middle-aged subjects. *Ann Med* 2010; 42:131–137.

- Pandolfi A, Cetrullo D, Polishuck R, Alberta MM, Calafiore A, Pellegrini G, Vita-colonna E, Capani F, Consoli A. Plasminogen activator inhibitor type 1 is increased in the arterial wall of type II diabetic subjects. *Arter Thromb Vasc Biol* 2001; 21:1378–1382.
- Paolisso G, Tagliamonte MR, Galderisi M, Zito GA, Petrocelli A, Carella C, de Divitiis O, Varricchio M. Plasma leptin level is associated with myocardial wall thickness in hypertensive insulin-resistant men. *Hypertension* 1999; 34:1047–1052.
- Parr SK, Clark AJ, Bian A, Shintani AK, Wickersham NE, Ware LB, Ikizler TA, Siew ED. Urinary L-FABP predicts poor outcomes in critically ill patients with early acute kidney injury. *Kidney Int* 2015; 87:640–648.
- Parthasarathy S, Raghavamenon A, Garelnabi MO, Santanam N. Oxidized low-density lipoprotein. *Methods Mol Biol* 2010; 610:403–417.
- Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000; 102:2165–2168.
- Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, Smith SA. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 2003; 300:472–476.
- Patti GJ, Yanes O, Siuzdak G. Metabolomics: the apogee of the omic trilogy. *Nat Rev Mol Cell Biol* 2012; 13:263–269.
- Pauca AL, O'Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* 2001; 38:932–937.
- Peco-Antić A, Ivanišević I, Vulićević I, Kotur-Stevuljević J, Ilić S, Ivanišević J, Miljković M, Kocev N. Biomarkers of acute kidney injury in pediatric cardiac surgery. *Clin Biochem* 2013; 46:1244–1251.
- Pedersen TR, Kjekshus J, Berg K, Haghfelt T, Faergeman O, Thorgeirsson G, Pyorala K, Miettinen T, Wilhelmsen L, Olsson AG, Wedel H, Kristianson K, Thomsen H, Nordero E, Thomsen B, Lyngborg K, Andersen GS, Nielsen F, Talleruphuus U, Mcnair A, Egstrup K, Simonsen EH, Simonsen I, Vejbychristensen H, Sommer L, Eidner PO, Klarholt E, Henriksen A, Mellempgaard K, Launbjerg J, Freuergaard P, Nielsen L, Madsen EB, Ibsen H, Andersen U, Enemark H, Haarbo J, Martinsen B, Dahlstrom CG, Thyrring L, Thomassen K, Jensen G, Rasmussen SL, Skov N, Hansen KN, Larsen ML, Haastrup B, Hjaere I, Thuroe A, Et A. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; 344:1383–1389.
- Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syväne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F; European Association for Cardiovascular Prevention & Rehabilitation (EACPR); ESC Committee for Practice Guidelines (CPG). European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). *Eur Heart J* 2012; 33:1635–1701.
- Peters M, Jacobs S, Ehlers M, Vollmer P, Müllberg J, Wolf E, Brem G, Meyer zum Büschenfelde KH, Rose-John S. The function of the soluble interleukin 6 (IL-6) receptor in vivo: sensitization of human soluble IL-6 receptor transgenic mice towards IL-6 and prolongation of the plasma half-life of IL-6. *J Exp Med* 1996; 183:1399–1406.

- Philippova M, Resink T, Erne P, Bochkov V. Oxidised phospholipids as biomarkers in human disease. *Swiss Med Wkly* 2014; 144:w14037. doi: 10.4414/smw.2014.14037.
- Pineda J, Marín F, Marco P, Roldán V, Valencia J, Ruiz-Nodar JM, Sogorb F, Lip GYH. Premature coronary artery disease in young (age < 45) subjects: interactions of lipid profile, thrombophilic and haemostatic markers. *Int J Cardiol* 2009; 136:222–225.
- Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc Natl Acad Sci U S A* 1987; 84:2995–2998.
- Rasmiena AA, Ng TW, Meikle PJ. Metabolomics and ischaemic heart disease. *Clin Sci* 2013; 124:289–306.
- Reilly MP, Iqbal N, Schutta M, Wolfe ML, Scally M, Localio AR, Rader DJ, Kimmel SE. Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. *J Clin Endocrinol Metab* 2004; 89:3872–3878.
- Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 2005; 111:932–939.
- Reiner Ž, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, Agewall S, Alegria E, Chapman MJ, Durrington P, Erdine S, Halcox J, Hobbs R, Kjekshus J, Filardi PP, Riccardi G, Storey RF, Wood D. ESC/EAS Guidelines for the management of dyslipidaemias. *Eur Heart J* 2011; 32:1769–1818.
- Rice TW, Lumsden AB. Optimal medical management of peripheral arterial disease. *Vasc Endovasc Surg* 2006; 40:312–327.
- Ridker PM, Rifai N, Stampfer M, Hennekens C. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000a; 101:1767–1772.
- Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000b; 342:836–843.
- Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1 β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am Heart J* 2011; 162:597–605.
- Ridker PM. Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT). *J Thromb Haemost* 2009a; 7:332–339.
- Ridker PM. C-reactive protein: eighty years from discovery to emergence as a major risk marker for cardiovascular disease. *Clin Chem* 2009b; 55:209–215.
- Ridker PM. From C-reactive protein to interleukin-6 to interleukin-1: moving upstream to identify novel targets for atheroprotection. *Circ Res* 2016; 118:145–156.
- Rietveld A, Simons K. The differential miscibility of lipids as the basis for the formation of functional membrane rafts. *Biochim Biophys Acta* 1998; 1376:467–479.
- Rietzschel ER, Langlois M, De Buyzere ML, Segers P, De Bacquer D, Bekaert S, Cooman L, Van Oostveldt P, Verdonck P, De Backer GG, Gillebert TC. Oxidized low-density lipoprotein cholesterol is associated with decreases in cardiac function independent of vascular alterations. *Hypertension* 2008; 52:535–541.
- Rizza S, Copetti M, Rossi C, Cianfarani MA, Zucchelli M, Luzi A, Pecchioli C, Porzio O, Di Cola G, Urbani A, Pellegrini F, Federici M. Metabolomics signature

- improves the prediction of cardiovascular events in elderly subjects. *Atherosclerosis* 2014; 232:260–264.
- Rizza S, Gigli F, Galli A, Michelini B, Lauro D, Lauro R, Federici M. Adiponectin isoforms in elderly patients with or without coronary artery disease. *J Am Geriatr Soc* 2010; 58:702–706.
- Roark EF, Keene DR, Haudenschild CC, Godyna S, Little CD, Argraves WS. The association of human fibulin-1 with elastic fibers: an immunohistological, ultrastructural, and RNA study. *J Histochem Cytochem* 1995; 43:401–411.
- Robless PA, Okonko D, Lintott P, Mansfield AO, Mikhailidis DP, Stansby GP. Increased platelet aggregation and activation in peripheral arterial disease. *Eur J Vasc Endovasc Surg* 2003; 25:16–22.
- Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med* 2014; 371:2383–2393.
- Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, Umans JG, Howard B V. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. *Hypertension* 2007; 50:197–203.
- Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int J Biol Sci* 2012; 8:1237–1247.
- Ross R. Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999; 340:115–126.
- Rossignol P, Luttun A, Martin-Ventura JL, Lupu F, Carmeliet P, Collen D, Angles-Cano E, Lijnen HR. Plasminogen activation: a mediator of vascular smooth muscle cell apoptosis in atherosclerotic plaques. *J Thromb Haemost* 2006; 4:664–670.
- Rudic RD, Sessa WC. Nitric oxide in endothelial dysfunction and vascular remodeling: clinical correlates and experimental links. *Am J Hum Genet* 1999; 64:673–677.
- Rus HG, Vlaicu R, Niculescu F. Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. *Atherosclerosis* 1996; 127:263–271.
- Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, Ito K, Sharma S, Ramadesikan S, Lee M, Briskin R, De Jager PL, Ngo TT, Radlinski M, Dear JW, Park KB, Betensky R, Krolewski AS, Bonventre JV. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *J Am Soc Nephrol* 2014; 25:2177–2186.
- Safar ME, Chamiot-Clerc P, Dagher G, Renaud JF. Pulse pressure, endothelium function, and arterial stiffness in spontaneously hypertensive rats. *Hypertension* 2001; 38:1416–1421.
- Safar ME, Blacher J, Pannier B, Guerin AP, Marchais SJ, Guyonvarc'h PM, London GM. Central pulse pressure and mortality in end-stage renal disease. *Hypertension* 2002; 39:735–738.
- Safar ME. Arterial stiffness and peripheral arterial disease. *Adv Cardiol* 2007a; 44:199–211.
- Safar ME. Arterial stiffness: a simplified overview in vascular medicine. *Adv Cardiol* 2007b; 44:1–18.
- Saitoh M, Osanai T, Kamada T, Matsunaga T, Ishizaka H, Hanada H, Okumura K. High plasma level of asymmetric dimethylarginine in patients with acutely exacerbated congestive heart failure: role in reduction of plasma nitric oxide level. *Heart Vessels* 2003; 18:177–182.

- Salgado JV, Souza FL, Salgado BJ. How to understand the association between cystatin C levels and cardiovascular disease: imbalance, counterbalance, or consequence? *J Cardiol* 2013; 62:331–335.
- Salonen JT, Nyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 1992; 86:803–811.
- Schäfer K, Halle M, Goeschen C, Dellas C, Pynn M, Loskutoff DJ, Konstantinides S. Leptin promotes vascular remodeling and neointimal growth in mice. *Arter Thromb Vasc Biol* 2004; 24:112–117.
- Scheffé H. *The Analysis of Variance*. New York: John Wiley & Sons; 1959.
- Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 1995; 270:26746–26749.
- Schillaci G, Pirro M, Mannarino MR, Pucci G, Savarese G, Franklin SS, Mannarino E. Relation between renal function within the normal range and central and peripheral arterial stiffness in hypertension. *Hypertension* 2006; 48:616–621.
- Schlatmann TJM, Becker AE. Histologic changes in the normal aging aorta: implications for dissecting aortic aneurysm. *Am J Cardiol* 1977; 39:13–20.
- Schmitz G, Ruebsaamen K. Metabolism and atherogenic disease association of lysophosphatidylcholine. *Atherosclerosis* 2010; 208:10–18.
- Schneiderman J, Sawdey MS, Keeton MR, Bordin GM, Bernstein EF, Dilley RB, Loskutoff DJ. Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries. *Proc Natl Acad Sci U S A* 1992; 89:6998–7002.
- Scholz A, Bladbjerg EM, Sidelmann JJ, Diederichsen ACP, Mickley H, Nybo M, Argraves WS, Marckmann P, Rasmussen LM. Plasma concentrations of extracellular matrix protein fibulin-1 are related to cardiovascular risk markers in chronic kidney disease and diabetes. *Cardiovasc Diabetol* 2013; 12:6. doi: 10.1186/1475-2840-12-6.
- Schooneman MG, Vaz FM, Houten SM, Soeters MR. Acylcarnitines: reflecting or inflicting insulin resistance? *Diabetes* 2013; 62:1–8.
- Segers P, Swillens A, Vermeersch S. Reservations on the reservoir. *J Hypertens* 2012; 30:676–678.
- Sehgel NL, Zhu Y, Sun Z, Trzeciakowski JP, Hong Z, Hunter WC, Vatner DE, Meininger GA, Vatner SF. Increased vascular smooth muscle cell stiffness: a novel mechanism for aortic stiffness in hypertension. *Am J Physiol Heart Circ Physiol* 2013; 305:1281–1287.
- Seino Y, Ikeda U, Ikeda M, Yamamoto K, Misawa Y, Hasegawa T, Kano S, Shimada K. Interleukin 6 gene transcripts are expressed in human atherosclerotic lesions. *Cytokine* 1994; 6:87–91.
- Sekas G, Patton GM, Lincoln EC, Robins SJ. Origin of plasma lysophosphatidylcholine: evidence for direct hepatic secretion in the rat. *J Lab Clin Med* 1985; 105:190–194.
- Shah SH, Sun JL, Stevens RD, Bain JR, Muehlbauer MJ, Pieper KS, Haynes C, Hauser ER, Kraus WE, Granger CB, Newgard CB, Califf RM, Newby LK. Baseline metabolomic profiles predict cardiovascular events in patients at risk for coronary artery disease. *Am Heart J* 2012; 163:844–850.e1.
- Shammas NW. Epidemiology, classification, and modifiable risk factors of peripheral arterial disease. *Vasc Heal Risk Manag* 2007; 3:229–234.

- Sharman JE, Davies JE, Jenkins C, Marwick TH. Augmentation index, left ventricular contractility, and wave reflection. *Hypertension* 2009; 54:1099–1105.
- Shaykhiev R, Bals R. Interactions between epithelial cells and leukocytes in immunity and tissue homeostasis. *J Leukoc Biol* 2007; 82:1–15.
- Shimada K, Mokuno H, Matsunaga E, Miyazaki T, Sumiyoshi K, Miyauchi K, Daida H. Circulating oxidized low-density lipoprotein is an independent predictor for cardiac event in patients with coronary artery disease. *Atherosclerosis* 2004; 174:343–347.
- Sies H. Oxidative stress: oxidants and antioxidants. *Exp Physiol* 1997; 82:291–295.
- Sigruener A, Kleber ME, Heimerl S, Liebis G, Schmitz G, Maerz W. Glycerophospholipid and sphingolipid species and mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *PLoS One* 2014; 9(1):e85724. doi: 10.1371/journal.pone.0085724.
- Sims TJ, Rasmussen LM, Oxlund H, Bailey AJ. The role of glycation cross-links in diabetic vascular stiffening. *Diabetologia* 1996; 39:946–951.
- Sinan UY, Canbolat IP, Baydar O, Oktay V, Imre G, Kocas C, Abaci O, Coskun U, Bostan C, Kilickesmez KO, Yildiz A, Kaya A, Gurmen T, Yigit Z. Relationship between increased serum resistin level and severity of coronary artery disease. *Angiology* 2014; 65:239–242.
- Singer E, Markó L, Paragas N, Barasch J, Dragun D, Müller DN, Budde K, Schmidt-Ott KM. Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. *Acta Physiol* 2013; 207:663–672.
- Smathers R, Petersen D. The human fatty acid-binding protein family: evolutionary divergences and functions. *Hum Genomics* 2011; 5:170–191.
- Smertka M, Wroblewska J, Suchojad A, Majcherczyk M, Jadamus-Niebroj D, Owsianka-Podlesny T, Brzozowska A, Maruniak-Chudek I. Serum and urinary NGAL in septic newborns. *Biomed Res Int* 2014; 2014:717318. doi: 10.1155/2014/717318.
- Smith A, Patterson C, Yarnell J, Rumley A, Ben-Shlomo Y, Lowe G. Which hemostatic markers add to the predictive value of conventional risk factors for coronary heart disease and ischemic stroke? The Caerphilly study. *Circulation* 2005; 112:3080–3087.
- Sobel BE, Woodcock-Mitchell J, Schneider DJ, Holt RE, Marutsuka K, Gold H. Increased plasminogen activator inhibitor type 1 in coronary artery atherectomy specimens from type 2 diabetic compared with nondiabetic patients: a potential factor predisposing to thrombosis and its persistence. *Circulation* 1998; 97:2213–2221.
- Solá E, Vayá A, España F, Castelló R, Ramón LA, Hernández-Mijares A, Vicente V, Estellés A. Plasminogen activator inhibitor-1 levels in severe and morbid obesity. Effect of weight loss and influence of 4G/5G polymorphism. *Thromb Res* 2008; 122:320–327.
- Solini A, Stea F, Santini E, Bruno RM, Duranti E, Taddei S, Ghiadoni L. Adipocytokine levels mark endothelial function in normotensive individuals. *Cardiovasc Diabetol* 2012; 11:103. doi: 10.1186/1475-2840-11-103.
- Spoto B, Mattace-Raso F, Sijbrands E, Pizzini P, Cutrupi S, D'Arrigo G, Tripepi G, Zoccali C, Mallamaci F. Resistin and all-cause and cardiovascular mortality: effect modification by adiponectin in end-stage kidney disease patients. *Nephrol Dial Transpl* 2013; 28 Suppl 4:iv181-7. doi: 10.1093/ndt/gft365.
- Stegemann C, Drozdov I, Shalhoub J, Humphries J, Ladroue C, Didangelos A, Baumert M, Allen M, Davies AH, Monaco C, Smith A, Xu Q, Mayr M. Comparative

- lipidomics profiling of human atherosclerotic plaques. *Circ Cardiovasc Genet* 2011; 4:232–242.
- Stegemann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, Menni C, Moayyeri A, Santer P, Rungger G, Spector TD, Willeit J, Kiechl S, Mayr M. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. *Circulation* 2014; 129:1821–1831.
- Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci U S A* 1984; 81:3883–3887.
- Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 2001a; 409:307–312.
- Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, Enders GH, Silberg DG, Wen X, Wu GD, Lazar M a. A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci U S A* 2001b; 98:502–506.
- Stevens LA, Schmid CH, Greene T, Zhang Y, Beck GJ, Froissart M, Hamm LL, Lewis JB, Mauer M, Navis GJ, Steffes MW, Eggers PW, Coresh J, Levey AS. Comparative performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 m². *Am J Kidney Dis* 2010; 56:486–495.
- Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC, Watson K, Wilson PWF. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014; 129:S1–S45.
- Subbaiah PV, Albers JJ, Chen CH, Bagdade JD. Low density lipoprotein-activated lysolecithin acylation by human plasma lecithin-cholesterol acyltransferase. Identity of lysolecithin acyltransferase and lecithin-cholesterol acyltransferase. *J Biol Chem* 1980; 255:9275–9280.
- Sung KC, Kang SM, Cho EJ, Park JB, Wild SH, Byrne CD. Ferritin is independently associated with the presence of coronary artery calcium in 12,033 men. *Arter Thromb Vasc Biol* 2012; 32:2525–2530.
- Surdacki A, Nowicki M, Sandmann J, Tsikas D, Boeger RH, Bode-Boeger SM, Kruszelnicka-Kwiatkowska O, Kokot F, Dubiel JS, Froelich JC. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. *J Cardiovasc Pharmacol* 1999; 33:652–658.
- Susantitaphong P, Siribamrungwong M, Doi K, Noiri E, Terrin N, Jaber BL. Performance of urinary liver-type fatty acid-binding protein in acute kidney injury: a meta-analysis. *Am J Kidney Dis* 2013; 61:430–439.
- Svensson-Färbom P, Ohlson Andersson M, Almgren P, Hedblad B, Engström G, Persson M, Christensson A, Melander O. Cystatin C identifies cardiovascular risk better than creatinine-based estimates of glomerular filtration in middle-aged individuals without a history of cardiovascular disease. *J Intern Med* 2014; 275:506–521.

- Syed Ikmal SI, Zaman Huri H, Vethakkan SR, Wan Ahmad WA. Potential biomarkers of insulin resistance and atherosclerosis in type 2 diabetes mellitus patients with coronary artery disease. *Int J Endocrinol* 2013; 2013:698567. doi: 10.1155/2013/698567.
- Takazoe K, Ogawa H, Yasue H, Sakamoto T, Soejima H, Miyao Y, Kawano H, Moriyama Y, Misumi K, Suefuji H, Kugiyama K, Yoshimura M. Increased plasminogen activator inhibitor activity and diabetes predict subsequent coronary events in patients with angina pectoris. *Ann Med* 2001; 33:206–212.
- Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013; 368:1575–84.
- Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995; 83:1263–1271.
- Taylor LA, Arends J, Hodina AK, Unger C, Massing U. Plasma lyso-phosphatidylcholine concentration is decreased in cancer patients with weight loss and activated inflammatory status. *Lipids Heal Dis* 2007;6:17.
- Thom S. Arterial structural modifications in hypertension. Effects of treatment. *Eur Heart J* 1997; 18 Suppl E:E2–E4.
- Thomsen M, Varbo A, Tybjaerg-Hansen A, Nordestgaard BG. Low nonfasting triglycerides and reduced all-cause mortality: a mendelian randomization study. *Clin Chem* 2014; 60:737–746.
- Thumser AE, Moore JB, Plant NJ. Fatty acid binding proteins: tissue-specific functions in health and disease. *Curr Opin Clin Nutr Metab Care* 2014; 17:124–129.
- Tillett WS, Francis T. Serological reactions in pneumonia with a non-protein somatic fraction of *Pneumococcus*. *J Exp Med* 1930; 52:561–571.
- Torregrosa I, Montoliu C, Urios A, Andrés-Costa MJ, Giménez-Garzó C, Juan I, Puchades MJ, Blasco ML, Carratalá A, Sanjuán R, Miguel A. Urinary KIM-1, NGAL and L-FABP for the diagnosis of AKI in patients with acute coronary syndrome or heart failure undergoing coronary angiography. *Heart Vessels* 2015; 30:703–711.
- Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM, Mitchell GF, Najjar SS, Nichols WW, Urbina EM, Weber T. Recommendations for improving and standardizing vascular research on arterial stiffness: a scientific statement from the American Heart Association. *Hypertension* 2015a; 66:698–722.
- Townsend RR. Arterial stiffness and chronic kidney disease: lessons from the Chronic Renal Insufficiency Cohort study. *Curr Opin Nephrol Hypertens* 2015b; 24:47–53.
- Tuominen JA, Ebeling P, Laquier FW, Heiman ML, Stephens T, Koivisto VA. Serum leptin concentration and fuel homeostasis in healthy man. *Eur J Clin Invest* 1997; 27:206–211.
- Ueland T, Svardal A, Øie E, Askevold ET, Nymoene SH, Bjørndal B, Dahl CP, Gullestad L, Berge RK, Aukrust P. Disturbed carnitine regulation in chronic heart failure – increased plasma levels of palmitoyl-carnitine are associated with poor prognosis. *Int J Cardiol* 2013; 167:1892–1899.
- Utzschneider KM, Carr DB, Tong J, Wallace TM, Hull RL, Zraika S, Xiao Q, Mistry JS, Retzlaff BM, Knopp RH, Kahn SE. Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. *Diabetologia* 2005; 48:2330–2333.

- Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol* 1992; 20:S60–S62.
- Van Bortel L, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank J, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso FUS, Protogerou AD, Schillaci G, Segers P, Vermeersch S, Weber T. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens* 2012; 30:445–448.
- Van Dijk RAJM, Bakker SJL, Scheffer PG, Heine RJ, Stehouwer CDA. Associations of metabolic variables with arterial stiffness in type 2 diabetes mellitus: focus on insulin sensitivity and postprandial triglyceridaemia. *Eur J Clin Invest* 2003; 33:307–315.
- Van Hinsbergh VW, Kooistra T, Van den Berg EA, Princen HM, Fiers W, Emeis JJ. Tumor necrosis factor increases the production of plasminogen activator inhibitor in human endothelial cells in vitro and in rats in vivo. *Blood* 1988; 72:1467–1473.
- Van Mourik JA, Lawrence DA, Loskutoff DJ. Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. *J Biol Chem* 1984; 259:14914–14921.
- Van Tits LJH, Stienstra R, Van Lent PL, Netea MG, Joosten LAB, Stalenhoef AFH. Oxidized LDL enhances pro-inflammatory responses of alternatively activated M2 macrophages: a crucial role for Krüppel-like factor 2. *Atherosclerosis* 2011; 214:345–349.
- Varbo A, Benn M, Tybjaerg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol* 2013; 61:427–436.
- Venugopal SK, Devaraj S, Jialal I. C-reactive protein decreases prostacyclin release from human aortic endothelial cells. *Circulation* 2003; 108:1676–1678.
- Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation* 2002; 106:1439–1441.
- Verma S, Li SH, Wang CH, Fedak PWM, Li RK, Weisel RD, Mickle DAG. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. *Circulation* 2003; 108:736–740.
- Vianey-Liaud C, Divry P, Gregersen N, Mathieu M. The inborn errors of mitochondrial fatty acid oxidation. *J Inherit Metab Dis* 1987; 10:159–200.
- Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010a; 55:1318–1327.
- Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J* 2010b; 31:1865–1871.
- Vlachopoulos C, Aznaouridis K, Stefanadis C. Aortic stiffness for cardiovascular risk prediction: just measure it, just do it! *J Am Coll Cardiol* 2014; 63:647–649.
- Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Hölm H, Ding EL, Johnson T, Schunkert H, Samani NJ, Clarke R, Hopewell JC, Thompson JF, Li M, Thorleifsson G, Newton-Cheh C, Musunuru K, Pirruccello JP, Saleheen D, Chen L, Stewart A, Schillert A, Thorsteinsdottir U, Thorgeirsson G, Anand S, Engert JC, Morgan T, Spertus J, Stoll M, Berger K, Martinelli N, Girelli D, McKeown PP, Patterson CC, Epstein SE, Devaney J, Burnett MS, Mooser V, Ripatti S, Surakka I, Nieminen MS, Sinisalo J, Lokki ML,

- Perola M, Havulinna A, de Faire U, Gigante B, Ingelsson E, Zeller T, Wild P, de Bakker PI, Klungel OH, Maitland-van der Zee AH, Peters BJ, de Boer A, Grobbee DE, Kamphuisen PW, Deneer VH, Elbers CC, Onland-Moret NC, Hofker MH, Wijmenga C, Verschuren WM, Boer JM, van der Schouw YT, Rasheed A, Frossard P, Demissie S, Willer C, Do R, Ordovas JM, Abecasis GR, Boehnke M, Mohlke KL, Daly MJ, Guiducci C, Burt NP, Surti A, Gonzalez E, Purcell S, Gabriel S, Marrugat J, Peden J, Erdmann J, Diemert P, Willenborg C, König IR, Fischer M, Hengstenberg C, Ziegler A, Buysschaert I, Lambrechts D, Van de Werf F, Fox KA, El Mokhtari NE, Rubin D, Schrezenmeier J, Schreiber S, Schäfer A, Danesh J, Blankenberg S, Roberts R, McPherson R, Watkins H, Hall AS, Overvad K, Rimm E, Boerwinkle E, Tybjaerg-Hansen A, Cupples LA, Reilly MP, Melander O, Mannucci PM, Ardissino D, Siscovick D, Elosua R, Stefansson K, O'Donnell CJ, Salomaa V, Rader DJ, Peltonen L, Schwartz SM, Altshuler D, Kathiresan S. Plasma HDL cholesterol and risk of myocardial infarction: A mendelian randomisation study. *Lancet* 2012; 380:572–580.
- Volanakis J. Human C-reactive protein: expression, structure, and function. *Mol Immunol* 2001; 38:189–197.
- Waikar S, Betensky R, Emerson S, Bonventre J. Imperfect gold standards for kidney injury biomarker evaluation. *J Am Soc Nephrol* 2012; 23:13–21.
- Wallert MA, Ackerman MJ, Kim D, Clapham DE. Two novel cardiac atrial K⁺ channels, IK.AA and IK.PC. *J Gen Physiol* 1991; 98:921–939.
- Walters GO, Miller FM, Worwood M. Serum ferritin concentration and iron stores in normal subjects. *J Clin Pathol* 1973; 26:770–772.
- Wanders RJA, Vreken P, Den Boer MEJ, Wijburg FA, Van Gennip AH, IJlst L. Disorders of mitochondrial fatty acyl-CoA β -oxidation. *J Inherit Metab Dis* 1999; 2(4):442–487.
- Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, He Y, Heim K, Campillos M, Holzapfel C, Thorand B, Grallert H, Xu T, Bader E, Huth C, Mittelstrass K, Döring A, Meisinger C, Gieger C, Prehn C, Roemisch-Margl W, Carstensen M, Xie L, Yamanaka-Okumura H, Xing G, Ceglarek U, Thiery J, Gianfranceschi G, Lickert H, Lin X, Li Y, Boeing H, Joost HG, de Angelis MH, Rathmann W, Suhre K, Prokisch H, Peters A, Meitinger T, Roden M, Wichmann HE, Pischon T, Adamski J, Illig T. Novel biomarkers for pre-diabetes identified by metabolomics. *Mol Syst Biol* 2012; 8:615. doi: 10.1038/msb.2012.43.
- Wang CY, Liu PY, Liao JK. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. *Trends Mol Med* 2008; 14:37–44.
- Wang G, Gong Y, Anderson J, Sun D, Minuk G, Roberts MS, Burczynski FJ. Antioxidative function of L-FABP in L-FABP stably transfected Chang liver cells. *Hepatology* 2005; 42:871–879.
- Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med* 2006; 355:2631–2639.
- Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011; 472:57–63.
- Wang Z, Tang WH, Buffa JA, Fu X, Britt EB, Koeth RA, Levison BS, Fan Y, Wu Y, Hazen SL. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. *Eur Heart J* 2014; 35(14):904–10.

- Watanabe M, Okada T. Lysophosphatidylcholine-induced myocardial damage is inhibited by pretreatment with poloxamer 188 in isolated rat heart. *Mol Cell Biochem* 2003; 248:209–215.
- Weber C, Erl W, Weber P. Enhancement of monocyte adhesion to endothelial cells by oxidatively modified low-density lipoprotein is mediated by activation of CD11b. *Biochem Biophys Res Commun* 1995; 206:621–628.
- Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R, Eber B. Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation* 2004; 109:184–189.
- Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Lamm G, Stark N, Rammer M, Eber B. Increased arterial wave reflections predict severe cardiovascular events in patients undergoing percutaneous coronary interventions. *Eur Heart J* 2005; 26:2657–2663.
- Weinbrenner T, Schröder H, Escuriol V, Fito M, Elosua R, Vila J, Marrugat J, Covas MI. Circulating oxidized LDL is associated with increased waist circumference independent of body mass index in men and women. *Am J Clin Nutr* 2006; 83:30–35.
- Wensley F, Gao P, Burgess S, Kaptoge S, Di Angelantonio E, Shah T, Engert JC, Clarke R, Davey-Smith G, Nordestgaard BG, Saleheen D, Samani NJ, Sandhu M, Anand S, Pepys MB, Smeeth L, Whittaker J, Casas JP, Thompson SG, Hingorani AD, Danesh J. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. *BMJ* 2011; 342:d548. doi: 10.1136/bmj.d548.
- Westerbacka J, Yki-Jarvinen H. Arterial stiffness and insulin resistance. *Semin Vasc Med* 2002; 2:157–164.
- Wilcox CS. Asymmetric dimethylarginine and reactive oxygen species: unwelcome twin visitors to the cardiovascular and kidney disease tables. *Hypertension* 2012; 59(2):375–381.
- Wildman RP, Mackey RH, Bostom A, Thompson T, Sutton-Tyrrell K. Measures of obesity are associated with vascular stiffness in young and older adults. *Hypertension* 2003; 42:468–473.
- Wilkinson IB, Cockcroft JR. Cholesterol, lipids and arterial stiffness. *Adv Cardiol* 2007; 44:261–277.
- Wilkinson IB, Prasad K, Hall I, Thomas A, MacCallum H, Webb D, Frenneaux M, Cockcroft JR. Increased central pulse pressure and augmentation index in subjects with hypercholesterolemia. *J Am Coll Cardiol* 2002; 39:1005–1011.
- Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol* 2000; 525 Pt 1:263–270.
- Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, Hughes AD, Thurston H, O'Rourke M. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 2006; 113:1213–1225.
- Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* 2006; 113:664–670.
- Windham BG, Griswold ME, Farasat SM, Ling SM, Carlson O, Egan JM, Ferrucci L, Najjar SS. Influence of leptin, adiponectin, and resistin on the association between abdominal adiposity and arterial stiffness. *Am J Hypertens* 2010; 23:501–507.

- Wolinsky H, Glagov S. Structural basis for the static mechanical properties of the aortic media. *Circ Res* 1964; 14:400–413.
- Wolk R, Berger P, Lennon RJ, Brilakis ES, Somers VK. Body mass index: a risk factor for unstable angina and myocardial infarction in patients with angiographically confirmed coronary artery disease. *Circulation* 2003; 108:2206–2211.
- Wong N. Epidemiological studies of CHD and the evolution of preventive cardiology. *Nat Rev Cardiol* 2014; 11:276–289.
- Woodman R, Chew G, Watts G. Mechanisms, significance and treatment of vascular dysfunction in type 2 diabetes mellitus: focus on lipid-regulating therapy. *Drugs* 2005; 65:31–74.
- Wu G, Morris SM. Arginine metabolism: nitric oxide and beyond. *Biochem J* 1998; 336:1–17.
- Wu I, Parikh C. Screening for kidney diseases: older measures versus novel biomarkers. *Clin J Am Soc Nephrol* 2008; 3:1895–1901.
- Wu J, McHowat J, Saffitz JE, Yamada KA, Corr PB. Inhibition of gap junctional conductance by long-chain acylcarnitines and their preferential accumulation in junctional sarcolemma during hypoxia. *Circ Res* 1993; 72:879–889.
- Würtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, Ghorbani A, Artati A, Wang Q, Tiainen M, Kangas AJ, Kettunen J, Kaikkonen J, Mikkilä V, Jula A, Kähönen M, Lehtimäki T, Lawlor DA, Gaunt TR, Hughes AD, Sattar N, Illig T, Adamski J, Wang TJ, Perola M, Ripatti S, Vasán RS, Raitakari OT, Gerszten RE, Casas JP, Chaturvedi N, Ala-Korpela M, Salomaa V. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation* 2015; 131:774–785.
- Wymann MP, Schneider R. Lipid signalling in disease. *Nat Rev Mol Cell Biol* 2008; 9:162–176.
- Xu A, Chan KW, Hoo RLC, Wang Y, Tan KCB, Zhang J, Chen B, Lam MC, Tse C, Cooper GJS, Lam KSL. Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *J Biol Chem* 2005; 280:18073–18080.
- Xu SY, Carlson M, Engström A, Garcia R, Peterson CG, Venge P. Purification and characterization of a human neutrophil lipocalin (HNL) from the secondary granules of human neutrophils. *Scand J Clin Lab Invest* 1994; 54:365–376.
- Yamamoto T, Noiri E, Ono Y, Doi K, Negishi K, Kamijo A, Kimura K, Fujita T, Kinukawa T, Taniguchi H, Nakamura K, Goto M, Shinozaki N, Ohshima S, Sugaya T. Renal L-type fatty acid – binding protein in acute ischemic injury. *J Am Soc Nephrol* 2007; 18:2894–2902.
- Yamauchi T, Kamon J, Ito Y, TsuchiLabela A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, UchLabela S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; 423:762–769.
- Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; 8:1288–1295.

- Yang CH, Chang CH, Chen TH, Fan PC, Chang SW, Chen CC, Chu PH, Chen YT, Yang HY, Yang CW, Chen YC. Combination of urinary biomarkers improves early detection of acute kidney injury in patients with heart failure. *Circ J* 2016; 80:1017–1023.
- Yang L, Brooks CR, Xiao S, Sabbisetti V, Yeung MY, Hsiao LL, Ichimura T, Kuchroo V, Bonventre J V. KIM-1-mediated phagocytosis reduces acute injury to the kidney. *J Clin Invest* 2015; 125:1620–1636.
- Ylä-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest* 1989; 84:1086–1095.
- Yoo JH, Lee SC. Elevated levels of plasma homocyst(e)ine and asymmetric dimethylarginine in elderly patients with stroke. *Atherosclerosis* 2001; 158:425–430.
- Youn JC, Kim C, Park S, Lee SH, Kang SM, Choi D, Son NH, Shin DJ, Jang Y. Adiponectin and progression of arterial stiffness in hypertensive patients. *Int J Cardiol* 2013; 163:316–319.
- Zagura M, Kals J, Kilk K, Serg M, Kampus P, Eha J, Soomets U, Zilmer M. Metabolomic signature of arterial stiffness in male patients with peripheral arterial disease. *Hypertens Res* 2015; 38:840–846.
- Zagura M, Kals J, Serg M, Kampus P, Zilmer M, Jakobson M, Unt E, Lieberg J, Eha J. Structural and biochemical characteristics of arterial stiffness in patients with atherosclerosis and in healthy subjects. *Hypertens Res* 2012; 35:1032–1037.
- Zeisel SH, Da Costa KA. Choline: an essential nutrient for public health. *Nutr Rev* 2009; 67:615–623.
- Zhang D, Shen X, Qi X. Resting heart rate and all-cause and cardiovascular mortality in the general population: a meta-analysis. *CMAJ* 2016; 188:53–63.
- Zhang R, Bai N, So J, Laher I, MacLeod KM, Rodrigues B. The ischemic metabolite lysophosphatidylcholine increases rat coronary arterial tone by endothelium-dependent mechanisms. *J Mol Cell Cardiol* 2009; 47:112–120.
- Zhang R, Brennan ML, Shen Z, MacPherson JC, Schmitt D, Molenda CE, Hazen SL. Myeloperoxidase functions as a major enzymatic catalyst for initiation of lipid peroxidation at sites of inflammation. *J Biol Chem* 2002; 277:46116–46122.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372:425–432.
- Zhao L, Song Y, Dong P, Li Z, Yang X, Wang S. Brachial pulse pressure and cardiovascular or all-cause mortality in the general population: a meta-analysis of prospective observational studies. *J Clin Hypertens* 2014; 16:678–685.
- Zhu Y, Zhang HP, Wang YC, Ren TT, Li J, Xu ML, Wang XQ, Liu FC, Lau A, Wen YF. Serum cystatin C level is associated with carotid intima-media thickening and plaque. *Scand J Clin Lab Invest* 2015; 75:265–72.
- Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto FA, Tripepi G, Malatino LS, Cataliotti A, Bellanuova I, Fermo I, Frölich JC, Böger RH. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 2001; 358:2113–2117.

SUMMARY IN ESTONIAN

Arterite jäikuse metabooliline profiil ja varajase neerukahjustuse biomarkerid ateroskleroosi korral

Suure ja keskmise läbimõõduga elastset tüüpi arterite võime kontraheeruda ja lõõgastuda toimib füsioloogiliselt olulise puhvrina, mis muundab südamsüklilist tingitud kõrge energiaga pulsatiivse verevoolu pidevaks ja ühtlaseks verevooluks, kaitstes seeläbi organite kapillaaristikku mehhaanilise kahjustuse eest. Ateroskleroosi korral (nt. stabiilse stenokardia ja alajäsemete arterite ateroskleroosiga haigetel) on arterid jäigenenud, s.t vähenenud on arterite võime vere rõhu tõusu mõjul laieneda. Suurenenud arterite jäikus viib muuhulgas südame vasaku vatsakese järeлкоormuse tõusu, hüpertroofia ning hapnikuvajaduse suurenemiseni. Arteriaalse jäikuse hindamise kuldstandardiks peetakse tänapäeval pulsiline leviku kiiruse registreerimist aordis. Pulsiline leviku kiirus aordis, st. aordi jäikus, ennustab sõltumatult üld- ja kardiovaskulaarset suremust nii erinevates haigusgruppides kui ka üldrahvastikus.

Muutuseid arterite struktuuris ja funktsioonis seostatakse ka neerufunktsiooni langusega. Kuna kliinilises töös laialdaselt kasutatav seerumi kreatiniini tase on suhteliselt hiline (48–72t) ja vähespetsiifiline neerukahjustuse indikaator, püütakse leida uusi biomarkereid, mis aitaksid neerukahjustust tuvastada võimalikult varajases etapis. Enimuuritud varajase neerukahjustuse uute biomarkerite hulka kuuluvad muuhulgas neutrofiilide želatinaasiga seotud lipokaliin (NGAL), neerukahjustuse molekul-1 (KIM-1), maksa-tüüpi rasvhappeid siduv valk (L-FABP), fibuliin-1 (FBLN-1) ja tsüstatiin C (CysC). Varasemad uuringud on näidanud arterite jäikuse sõltumatut seost kroonilise neerupuudulikkusega. Siiski on veel vähe andmeid arterite jäigenemise rolli kohta neerukahjustuse tekkes ja arengus. Uute varajase neerukahjustuse biomarkerite ja arterite struktuuri- ja funktsiooni parameetrite vaheliste seoste tuvastamine ja analüüsimine võimaldab laiendada teadmisi selles valdkonnas.

Südame- ja veresoonekonna haiguste riski tõstavad lisaks langenud neerufunktsioonile ja hemodünaamika muutustele ka süsteemsed metaboolsed nihked organismis. Metaboolomika on kiiresti arenev teadusharu, mis võimaldab bioloogilise materjali madalmolekulaarsete ühendite (nt aminohapped, peptiidid, lipiidid, süsivesikud, nukleiinhapped, rasvhapped) profiili tuvastamise kaudu hinnata erinevate haigustega kaasnevaid spetsiifilisi ainevahetuslikke muutuseid ehk nn metaboolset sõrmejälge. Uudsete metaboolomiliste andmete sidumine juba valideeritud ja kliiniliselt oluliste funktsionaalsete ja biokeemiliste näitajatega võib viia nii uute tundlikumate ja spetsiifilisemate biomarkerite kui ka tõhusate terapeutiliste sihtmärkide tuvastamiseni. Madalmolekulaarsete ühendite roll arterite jäigenemises ja nende seosed teiste hemodünaamiliste parameetritega ateroskleroosiga haigetel on siiani suuresti teadmata.

Uurimuse eesmärgid

Käesoleva töö üldiseks eesmärgiks oli analüüsida arterite jäikuse ja varajase neerukahjustuse biomarkerite omavahelisi seoseid ning tuvastada arterite jäikuse madalmolekulaarsete ühendite profiil stabiilse stenokardiaga haigetel, alajäsemete arterite ateroskleroosiga haigetel ning kliiniliselt tervetel uuritavatel.

Uurimuse täpsed eesmärgid olid järgmised:

1. Määrata FBLN-1 seerumi taset ja kirjeldada selle seoseid arterite jäikuse ja põletiku näitajatega stabiilse stenokardiaga haigetel, alajäsemete arterite ateroskleroosiga haigetel ja kliiniliselt tervetel uuritavatel.
2. Mõõta NGAL ja CysC seerumi tasemeid, L-FABP ja KIM-1 uriini tasemeid ning hinnata nende seoseid arterite jäikuse ja põletiku näitajatega stabiilse stenokardiaga haigetel (glomerulaarfiltratsioon ≥ 60 mL/min/1.73 m²) ja kliiniliselt tervetel uuritavatel.
3. Uurida atsüülkarnitiinide seerumi tasemete seoseid aordi jäikuse, hemodünaamika ja põletiku näitajatega stabiilse stenokardiaga patsientidel ja kliiniliselt tervetel uuritavatel.
4. Hinnata fosfatidüülkoliinide ja lüsofosfatidüülkoliinide seerumi tasemete seoseid arterite jäikuse, hemodünaamika ja endoteeli düsfunktsiooniga stabiilse stenokardiaga haigetel ja kliiniliselt tervetel uuritavatel.
5. Tuvastada ja analüüsida veresoonte funktsionaalsuse ning biokeemilise ja metaboolmilise profiili erinevusi ja sarnasusi stabiilse stenokardiaga haigete ja alajäsemete arterite ateroskleroosiga haigete vahel.

Uuringute meetodid

Uuringulusteks olid 52 stabiilse stenokardiaga meespatsienti, 38 alajäsemete arterite ateroskleroosiga meespatsienti (II – IV staadium Fontaine'i järgi) ja 41 kliiniliselt tervet meest. Arterite jäikust hinnati pulsiline kiiruse ja pulsiline analüüsi kaudu Tartu Ülikooli Kardioloogiakliiniku Endoteeli Keskuses. Neerukahjustuse- ja põletiku biomarkerite ning madalmolekulaarsete ühendite seerumi/uriini tasemed määrati Tartu Ülikooli bio- ja siirdemeditiini instituudi biokeemia osakonnas ja Sihtasutus Tartu Ülikooli Kliinikumi Ühendlaboris.

Tulemused ja järeldused

1. FBLN-1 seerumi tase oli alajäsemete arterite ateroskleroosiga haigetel võrreldes kliiniliselt tervete uuritavatega oluliselt tõusnud ning sõltumatult seotud arterite jäikusega. Stabiilse stenokardiaga haigete ja kliiniliselt tervete uuritavate hulgas ülalmainitud seost ei esinenud. Seerumi FBLN-1 tase oli ateroskleroosiga haigetel seotud ka kroonilise põletikuga, mistõttu võib see valk tulevikus leida kasutust süsteemse vaskulaarse kahjustuse biomarkerina.
2. L-FABP ja KIM-1 uriini tasemed ja NGAL ning CysC seerumi tasemed ei olnud stabiilse stenokardiaga haigete (glomerulaarfiltratsioon ≥ 60 mL/min/1.73 m²) ja

kliiniliselt tervete uuritavate vahel oluliselt erinevad. Küll aga olid L-FABP ja KIM-1 uriini tasemed haigete grupis sõltumatult seotud aordi jäikusega, mis võib viidata arterite funktsiooni languse ja varajase neerukahjustuse omavahelistele seostele.

3. Stabiilse stenokardiaga haigetel oli aordi jäikus sõltumatult seotud keskmise- ja pikaahelaliste atsüülkarnitiinide kõrgema seerumi tasemega. Lisaks korreleerusid selles uuritavate grupis seerumi palmitoüülkarnitiin ja karnitiin palmitoüültransferaas I mitmete hemodünaamiliste parameetritega. Üldmainitud tulemused viitavad, et lisaks traditsioonilistele lipiidide ainevahetuse biomarkeritele võivad atsüülkarnitiinid tulevikus kasutust leida veresoonte kahjustuse uudsete indikaatoritena.
4. Mitmete fosfatidüülkoliinide ja lüsofosfatidüülkoliinide madalamad seerumi tasemed olid stabiilse stenokardiaga haigetel ja alajäsemete arterite ateroskleroosiga haigetel, aga mitte kliiniliselt tervetel uuritavatel, seotud suurenenud arterite jäikuse, südame löögisageduse ja endoteeli düsfunktsiooniga. Seega võivad need tulemused vähemalt osaliselt selgitada varasemates uuringutes leitud seoseid kõnealuste lipiidide madalama seerumi/plasma taseme ja kõrgema südame- ja veresoonekonna haiguste riski vahel.
5. Stabiilse stenokardiaga haigete ja alajäsemete arterite ateroskleroosiga haigete põletiku biomarkerite ja metaboolilise profiili seoste vahel teiste biokeemiliste- ja veresoonte funktsionaalsuse parameetritega esines olulisi erinevusi. Selle põhjuseks võivad muuhulgas olla nii ateroskleroosi ulatuse ja raskusastme kui ka südame- ja veresoonekonna haiguste riskitegurite levimuse erinevused kahe haigete grupi vahel.

ACKNOWLEDGEMENTS

This research was supported by a grant of the Estonian Science Foundation (No. 9094), by Personal Research Funding (GMVBS1169P), by Institutional Research Funding (Nos. IUT20-42, IUT2-7) from the Estonian Ministry of Education and by the European Union through the European Regional Development Fund (Project No. 2014-2020.4.01.15-0012).

It is my pleasure to express my sincerest thanks and appreciation to those who have contributed to the present thesis:

- Dr. Jaak Kals, my principal supervisor and friend, for his enthusiastic encouragement and useful critiques, but foremost for his willingness to give his time so generously;
- Prof. Mihkel Zilmer, my supervisor, for his kind support throughout my thesis with his knowledge, expertise and endless optimism;
- Prof. Jaan Eha, my supervisor, for inviting me to do research and for his unrelenting support;
- Dr. Kaspar Tootsi, my fellow PhD student, co-author and friend, for our enjoyable conversations and scientific discussions, as well as for helping me keep a sense of humor;
- Mrs. Anneli Piir, my co-author, and Mrs. Kersti Zilmer, for the measurement of biochemical markers of inflammation and oxidative stress;
- Mr. Aigar Ottas, my co-author, for the measurement of low molecular weight metabolites;
- Dr. Meelis Jakobson, my co-author, for his assistance in the calculation of angiographic score;
- Prof. Vallo Volke and Prof. Vallo Tillmann, for reviewing this thesis;
- Dr. Priit Kampus, for his words of encouragement and for his kind invitations to several clinical conferences;
- Mrs. Sille Tamm, for finding perfect solutions to everyday household problems during my PhD studies;
- Ms. Riina Kaur for her good humour and technical help;
- Dr. Martin Serg, Dr. Maksim Zagura, Dr. Erik Salum, and Dr. Piibe Muda for offering their friendly advice and support on all kinds of issues;
- Dr. Tuuli Teeäär, Dr. Aet Saar, Dr. Teele Kepler, Dr. Karl Kuusik, and Dr. Priit Pauklin, my fellow PhD students, for carrying the torch of science forward;
- Mr. Mart Kals, for statistical assistance;
- Mrs. Ester Jaigma, for the linguistic revision of my manuscripts;
- The staff from the Department of Biochemistry, for their kind support;
- The staff from the Department of Cardiology and from the Department of Vascular Surgery, for their technical assistance;
- All subjects who participated in our studies, for giving their time and energy to make this thesis possible;

- Dr. Carmel M McEniery, Dr. Thomas Hiemstra, and Prof. Ian B Wilkinson, from the University of Cambridge, for their warm encouragement and supervision during my studies in the Division of Experimental Medicine and Immunotherapeutics;
- Gert, Taavi-Peeter, Dirk, Oleg, and Mihkel, my friends, for the good times and music.

The present thesis is dedicated to my beloved family, my precious Kärt and our unborn child.

PUBLICATIONS

CURRICULUM VITAE

Name: Kaido Paapstel
Date of birth: April 8, 1987, Tallinn, Estonia
Citizenship: Estonian
Phone: +372 731 8457
E-mail: kaido.paapstel@kliinikum.ee

Education:

1994–2006 Tallinn Mustamäe Gymnasium, silver medal
2006–2012 University of Tartu, Faculty of Medicine, Medicine
2012–2016 University of Tartu, Faculty of Medicine, PhD studies
2016– University of Tartu, Faculty of Medicine, Residency in Cardiology

Professional employment:

2015–2016 Visiting Researcher, University of Cambridge, Cambridge, United Kingdom (5 months)

Special courses:

2013 International Symposium “New ways in the management of hypertension: diagnostics of arterial stiffness and choice of 3rd-generation beta blockers in combination therapy”, Riga, Latvia
2013 European Society of Cardiology Educational Courses 2013, Tallinn, Estonia
2014 International Conference “Artery 14”, Maastricht, Netherlands
2015 Royal College of Physicians/CMS Regional Meeting: Acute & General Internal Medicine, Cambridge, United Kingdom
2016 84th European Atherosclerosis Society Congress, Innsbruck, Austria

Scientific work:

My principal research interests lie in exploring the links between arterial stiffness, renal damage, low-grade inflammation and metabolomics. Seven scientific articles published/accepted in international peer-reviewed journals. Three presentations at international scientific conferences.

Publications in international peer-reviewed journals:

Paapstel K, Zilmer M, Eha J, Tootsi K, Piir A, Kals J. Association between fibulin-1 and aortic augmentation index in male patients with peripheral arterial disease. *Eur J Vasc Endovasc Surg* 2016; 51(1): 76–82.
Paapstel K, Zilmer M, Eha J, Tootsi K, Piir A, Kals J. Early biomarkers of renal damage in relation to arterial stiffness and inflammation in male coronary artery disease patients. *Kidney Blood Press Res* 2016;41(4): 488–97.

- Paapstel K, Kals J, Eha J, Tootsi K, Ottas A, Piir A, Zilmer M. Metabolomic profiles of lipid metabolism, arterial stiffness and hemodynamics in male coronary artery disease patients. *IJC Metab Endocr* 2016; 11: 13–18.
- Zagura M; Kals J; Paapstel K; Serg M; Kampus P; Eha J; Zilmer M. Response to “Estimation of pulse wave velocity in patients with peripheral artery disease: a word of caution”. *Hypertens Res* 2016, 39(8): 618–619.
- Tootsi K; Kals J; Zilmer M; Paapstel K; Märtson A. Severity of osteoarthritis is associated with increased arterial stiffness. *Int J Rheumatol* 2016; 2016:6402963. doi: 10.1155/2016/6402963.
- Tootsi K; Märtson A; Zilmer M; Paapstel K; Kals J. Increased arterial stiffness in patients with end-stage osteoarthritis: a case-control study. *BMC Musculoskelet Disord* 2016; 17:335. doi: 10.1186/s12891-016-1201-x.
- Kaldur T; Unt E; Ööpik V; Zilmer M; Eha J; Paapstel K; Kals J. The acute effects of passive heat exposure on arterial stiffness, oxidative stress and inflammation. *Medicina* 2016, doi: 10.1016/j.medic.2016.06.001.

ELULOOKIRJELDUS

Nimi: Kaido Paapstel
Sünniaeg: 8. aprill 1987, Tallinn, Eesti
Kodakondsus: Eesti
Telefon: +372 731 8457
E-post: kaido.paapstel@kliinikum.ee

Haridus:
1994-2006 Tallinna Mustamäe Gümnaasium, hõbemedal
2006-2012 Arstiteadus, Tartu Ülikooli arstiteaduskond
2012-2016 Doktorantuur, Tartu Ülikooli arstiteaduskond
2016– Residentuur (kardioloogia), Tartu Ülikooli arstiteaduskond

Erialane teenistuskäik:

2015-2016 Kõlalisidoktorant, Cambridge'i Ülikool, Cambridge, Suurbritannia (5 kuud)

Erialane täiendus:

2013 Rahvusvaheline sümposium "New ways in the management of hypertension: diagnostics of arterial stiffness and choice of 3rd-generation beta blockers in combination therapy", Riia, Läti
2013 Rahvusvaheline konverents "European Society of Cardiology Educational Courses 2013", Tallinn, Eesti
2014 Rahvusvaheline konverents "Artery 14", Maastricht, Holland
2015 Sümposium "Royal College of Physicians/CMS Regional Meeting: Acute & General Internal Medicine", Cambridge, Suurbritannia
2016 Rahvusvaheline konverents "84th European Atherosclerosis Society Congress", Innsbruck, Austria

Teadustöö:

Minu teadustöö põhisuundadeks on arterite jäikuse, neerukahjustuse, süsteemse kroonilise põletiku ja madalmolekulaarsete ühendite spektri vaheliste seoste tuvastamine ja analüüs.

Ilmunud on 7 teaduslikku artiklit rahvusvahelistes eelretsenseeritavates ajakirjades. 3 ettekannet rahvusvahelistel teaduslikel konverentsidel.

Artiklid rahvusvahelistes eelretsenseeritavates ajakirjades:

Paapstel K, Zilmer M, Eha J, Tootsi K, Piir A, Kals J. Association between fibulin-1 and aortic augmentation index in male patients with peripheral arterial disease. *Eur J Vasc Endovasc Surg* 2016; 51(1): 76–82.

Paapstel K, Zilmer M, Eha J, Tootsi K, Piir A, Kals J. Early biomarkers of renal damage in relation to arterial stiffness and inflammation in male coronary artery disease patients. *Kidney Blood Press Res* 2016;41(4): 488–97.

- Paapstel K, Kals J, Eha J, Tootsi K, Ottas A, Piir A, Zilmer M. Metabolomic profiles of lipid metabolism, arterial stiffness and hemodynamics in male coronary artery disease patients. *IJC Metab Endocr* 2016; 11: 13–18.
- Zagura M; Kals J; Paapstel K; Serg M; Kampus P; Eha J; Zilmer M. Response to “Estimation of pulse wave velocity in patients with peripheral artery disease: a word of caution”. *Hypertens Res* 2016, 39(8): 618–619.
- Tootsi K; Kals J; Zilmer M; Paapstel K; Märtson A. Severity of osteoarthritis is associated with increased arterial stiffness. *Int J Rheumatol* 2016; 2016:6402963. doi: 10.1155/2016/6402963.
- Tootsi K; Märtson A; Zilmer M; Paapstel K; Kals J. Increased arterial stiffness in patients with end-stage osteoarthritis: a case-control study. *BMC Musculoskelet Disord* 2016; 17:335. doi: 10.1186/s12891-016-1201-x.
- Kaldur T; Unt E; Ööpik V; Zilmer M; Eha J; Paapstel K; Kals J. The acute effects of passive heat exposure on arterial stiffness, oxidative stress and inflammation. *Medicina* 2016, doi: 10.1016/j.medic.2016.06.001.

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaros.** The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer.** Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar.** Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik.** Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu.** Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar.** Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus.** Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar.** Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm.** On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross.** Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo.** Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik.** The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa.** Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk.** Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar.** Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi.** The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar.** Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang.** The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak.** Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.

20. **Tõnis Karki.** Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.
21. **Reet Mändar.** Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel.** Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik.** Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk.** Stress due to sleep deprivation: focus on GABA_A receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets.** Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik.** Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi.** Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo.** Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber.** *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla.** Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk.** Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf.** Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv.** Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi.** Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva.** Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin.** Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev.** Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik.** Thyroid hormone control over β -adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto.** Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar.** Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge.** Humoral immune responses to allergens in early childhood. Tartu, 1998.

42. **Heli Grünberg.** The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.
43. **Epp Sepp.** Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots.** Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe.** Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv.** Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan.** Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik.** Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand.** Autoantibodies against dehydrogenases of α -ketoacids. Tartu, 1999.
50. **Toomas Marandi.** Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask.** Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson.** Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar.** Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl.** Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap.** Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg.** *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro.** Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi.** Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann.** Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke.** Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves.** Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda.** Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist.** Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.

64. **Jana Kivastik.** Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptoms, reference values for dynamic spirometry. Tartu, 2000.
65. **Karin Kull.** Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos.** Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova.** Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda.** Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner.** *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi.** Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla.** Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas.** Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk.** Selection of medicinal plants and intestinal lactobacilli as antimicrobial components for functional foods. Tartu, 2002.
74. **Aet Lukmann.** Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen.** Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar.** Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep.** Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes.** Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu.** Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi.** Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa.** Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene.** *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.

83. **Helgi Kolk.** Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.
84. **Helena Soomer.** Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.
85. **Kersti Oselin.** Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.
86. **Jaan Soplepmann.** Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.
87. **Margot Peetsalu.** Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.
88. **Kersti Klaamas.** Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.
89. **Pille Taba.** Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.
90. **Alar Veraksitš.** Characterization of behavioural and biochemical phenotype of cholecystikinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.
91. **Ingrid Kalev.** CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.
92. **Lumme Kadaja.** Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.
93. **Aive Liigant.** Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.
94. **Andres, Kulla.** Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.
95. **Mari Järvelaid.** Health damaging risk behaviours in adolescence. Tartu, 2004.
96. **Ülle Pechter.** Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.
97. **Gunnar Tasa.** Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.
98. **Tuuli Käämbre.** Intracellular energetic unit: structural and functional aspects. Tartu, 2004.
99. **Vitali Vassiljev.** Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.

100. **Aune Rehema.** Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.
101. **Evelin Seppet.** Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.
102. **Eduard Maron.** Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.
103. **Marje Oona.** *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.
104. **Kersti Kokk.** Regulation of active and passive molecular transport in the testis. Tartu, 2005.
105. **Vladimir Järv.** Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.
106. **Andre Õun.** Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.
107. **Piibe Muda.** Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.
108. **Küllli Kingo.** The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.
109. **Mati Merila.** Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.
110. **Epp Songisepp.** Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.
111. **Tiia Ainla.** Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.
112. **Andres Sell.** Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.
113. **Tiia Tamme.** Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.
114. **Triine Annus.** Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.
115. **Tiia Voor.** Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.
116. **Priit Kasenõmm.** Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.
117. **Eva Zusinaite.** Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.
118. **Piret Kõll.** Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.
119. **Tiina Stelmach.** Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.

120. **Katrin Pudersell.** Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Küllil Jaako.** Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.
122. **Aare Märtsen.** Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld.** Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann.** Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar.** Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson.** Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus.** Mechanism of action of antidepressants: aspects of serotonergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder.** Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru.** Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand.** Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo.** Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo.** The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild.** Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik.** Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller.** Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik.** Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals.** Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus.** Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab.** Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom.** Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.

141. **Lea Pehme.** Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin.** The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik.** Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.
144. **Tarvo Rajasalu.** Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu.** Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik.** Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt.** Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets.** The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits.** Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam.** Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots.** Cationic regulation of Na-pump in the normal, Alzheimer's and CCK₂ receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp.** The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull.** Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre.** Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link.** Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre.** Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak.** Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich.** Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep.** Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk.** Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.

161. **Kaire Heilman**. Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel**. HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller**. Immune markers in major depression and in antidepressive treatment. Tartu, 2009.
164. **Siim Suutre**. The role of TGF- β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman**. Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa**. Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org**. Molecular function of the first PHD finger domain of Auto-immune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht**. Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu**. Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand**. Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull**. Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.
172. **Rael Laugesaar**. Stroke in children – epidemiology and risk factors. Tartu, 2010.
173. **Mark Braschinsky**. Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implementation of genetic analysis in everyday neurologic practice. Tartu, 2010.
174. **Kadri Suija**. Major depression in family medicine: associated factors, recurrence and possible intervention. Tartu, 2010.
175. **Jarno Habicht**. Health care utilisation in Estonia: socioeconomic determinants and financial burden of out-of-pocket payments. Tartu, 2010.
176. **Kristi Abram**. The prevalence and risk factors of rosacea. Subjective disease perception of rosacea patients. Tartu, 2010.
177. **Malle Kuum**. Mitochondrial and endoplasmic reticulum cation fluxes: Novel roles in cellular physiology. Tartu, 2010.
178. **Rita Teek**. The genetic causes of early onset hearing loss in Estonian children. Tartu, 2010.
179. **Daisy Volmer**. The development of community pharmacy services in Estonia – public and professional perceptions 1993–2006. Tartu, 2010.
180. **Jelena Lissitsina**. Cytogenetic causes in male infertility. Tartu, 2011.
181. **Delia Lepik**. Comparison of gunshot injuries caused from Tokarev, Makarov and Glock 19 pistols at different firing distances. Tartu, 2011.
182. **Ene-Renate Pähkla**. Factors related to the efficiency of treatment of advanced periodontitis. Tartu, 2011.

183. **Maarja Krass.** L-Arginine pathways and antidepressant action. Tartu, 2011.
184. **Taavi Lai.** Population health measures to support evidence-based health policy in Estonia. Tartu, 2011.
185. **Tiit Salum.** Similarity and difference of temperature-dependence of the brain sodium pump in normal, different neuropathological, and aberrant conditions and its possible reasons. Tartu, 2011.
186. **Tõnu Vooder.** Molecular differences and similarities between histological subtypes of non-small cell lung cancer. Tartu, 2011.
187. **Jelena Štšepetova.** The characterisation of intestinal lactic acid bacteria using bacteriological, biochemical and molecular approaches. Tartu, 2011.
188. **Radko Avi.** Natural polymorphisms and transmitted drug resistance in Estonian HIV-1 CRF06_cpx and its recombinant viruses. Tartu, 2011, 116 p.
189. **Edward Laane.** Multiparameter flow cytometry in haematological malignancies. Tartu, 2011, 152 p.
190. **Triin Jagomägi.** A study of the genetic etiology of nonsyndromic cleft lip and palate. Tartu, 2011, 158 p.
191. **Ivo Laidmäe.** Fibrin glue of fish (*Salmo salar*) origin: immunological study and development of new pharmaceutical preparation. Tartu, 2012, 150 p.
192. **Ülle Parm.** Early mucosal colonisation and its role in prediction of invasive infection in neonates at risk of early onset sepsis. Tartu, 2012, 168 p.
193. **Kaupo Teesalu.** Autoantibodies against desmin and transglutaminase 2 in celiac disease: diagnostic and functional significance. Tartu, 2012, 142 p.
194. **Maksim Zagura.** Biochemical, functional and structural profiling of arterial damage in atherosclerosis. Tartu, 2012, 162 p.
195. **Vivian Kont.** Autoimmune regulator: characterization of thymic gene regulation and promoter methylation. Tartu, 2012, 134 p.
196. **Pirje Hütt.** Functional properties, persistence, safety and efficacy of potential probiotic lactobacilli. Tartu, 2012, 246 p.
197. **Innar Tõru.** Serotonergic modulation of CCK-4- induced panic. Tartu, 2012, 132 p.
198. **Sigrid Vorobjov.** Drug use, related risk behaviour and harm reduction interventions utilization among injecting drug users in Estonia: implications for drug policy. Tartu, 2012, 120 p.
199. **Martin Serg.** Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension. Tartu, 2012, 156 p.
200. **Jaanika Kumm.** Molecular markers of articular tissues in early knee osteoarthritis: a population-based longitudinal study in middle-aged subjects. Tartu, 2012, 159 p.
201. **Kertu Rünkorg.** Functional changes of dopamine, endopioid and endocannabinoid systems in CCK2 receptor deficient mice. Tartu, 2012, 125 p.
202. **Mai Blöndal.** Changes in the baseline characteristics, management and outcomes of acute myocardial infarction in Estonia. Tartu, 2012, 127 p.

203. **Jana Lass.** Epidemiological and clinical aspects of medicines use in children in Estonia. Tartu, 2012, 170 p.
204. **Kai Truusalu.** Probiotic lactobacilli in experimental persistent *Salmonella* infection. Tartu, 2013, 139 p.
205. **Oksana Jagur.** Temporomandibular joint diagnostic imaging in relation to pain and bone characteristics. Long-term results of arthroscopic treatment. Tartu, 2013, 126 p.
206. **Katrin Sikk.** Manganese-ephedrone intoxication – pathogenesis of neurological damage and clinical symptomatology. Tartu, 2013, 125 p.
207. **Kai Blöndal.** Tuberculosis in Estonia with special emphasis on drug-resistant tuberculosis: Notification rate, disease recurrence and mortality. Tartu, 2013, 151 p.
208. **Marju Puurand.** Oxidative phosphorylation in different diseases of gastric mucosa. Tartu, 2013, 123 p.
209. **Aili Tagoma.** Immune activation in female infertility: Significance of autoantibodies and inflammatory mediators. Tartu, 2013, 135 p.
210. **Liis Sabre.** Epidemiology of traumatic spinal cord injury in Estonia. Brain activation in the acute phase of traumatic spinal cord injury. Tartu, 2013, 135 p.
211. **Merit Lamp.** Genetic susceptibility factors in endometriosis. Tartu, 2013, 125 p.
212. **Erik Salum.** Beneficial effects of vitamin D and angiotensin II receptor blocker on arterial damage. Tartu, 2013, 167 p.
213. **Maire Karelson.** Vitiligo: clinical aspects, quality of life and the role of melanocortin system in pathogenesis. Tartu, 2013, 153 p.
214. **Kuldar Kaljurand.** Prevalence of exfoliation syndrome in Estonia and its clinical significance. Tartu, 2013, 113 p.
215. **Raido Paasma.** Clinical study of methanol poisoning: handling large outbreaks, treatment with antidotes, and long-term outcomes. Tartu, 2013, 96 p.
216. **Anne Kleinberg.** Major depression in Estonia: prevalence, associated factors, and use of health services. Tartu, 2013, 129 p.
217. **Triin Eglit.** Obesity, impaired glucose regulation, metabolic syndrome and their associations with high-molecular-weight adiponectin levels. Tartu, 2014, 115 p.
218. **Kristo Ausmees.** Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status. Tartu, 2014, 125 p.
219. **Kristi Huik.** The influence of host genetic factors on the susceptibility to HIV and HCV infections among intravenous drug users. Tartu, 2014, 144 p.
220. **Liina Tserel.** Epigenetic profiles of monocytes, monocyte-derived macrophages and dendritic cells. Tartu, 2014, 143 p.
221. **Irina Kerna.** The contribution of *ADAM12* and *CILP* genes to the development of knee osteoarthritis. Tartu, 2014, 152 p.

222. **Ingrit Liiv.** Autoimmune regulator protein interaction with DNA-dependent protein kinase and its role in apoptosis. Tartu, 2014, 143 p.
223. **Liivi Maddison.** Tissue perfusion and metabolism during intra-abdominal hypertension. Tartu, 2014, 103 p.
224. **Krista Ress.** Childhood coeliac disease in Estonia, prevalence in atopic dermatitis and immunological characterisation of coexistence. Tartu, 2014, 124 p.
225. **Kai Muru.** Prenatal screening strategies, long-term outcome of children with marked changes in maternal screening tests and the most common syndromic heart anomalies in Estonia. Tartu, 2014, 189 p.
226. **Kaja Rahu.** Morbidity and mortality among Baltic Chernobyl cleanup workers: a register-based cohort study. Tartu, 2014, 155 p.
227. **Klari Noormets.** The development of diabetes mellitus, fertility and energy metabolism disturbances in a Wfs1-deficient mouse model of Wolfram syndrome. Tartu, 2014, 132 p.
228. **Liis Toome.** Very low gestational age infants in Estonia. Tartu, 2014, 183 p.
229. **Ceith Nikkolo.** Impact of different mesh parameters on chronic pain and foreign body feeling after open inguinal hernia repair. Tartu, 2014, 132 p.
230. **Vadim Brjalin.** Chronic hepatitis C: predictors of treatment response in Estonian patients. Tartu, 2014, 122 p.
231. **Vahur Metsna.** Anterior knee pain in patients following total knee arthroplasty: the prevalence, correlation with patellar cartilage impairment and aspects of patellofemoral congruence. Tartu, 2014, 130 p.
232. **Marju Kase.** Glioblastoma multiforme: possibilities to improve treatment efficacy. Tartu, 2015, 137 p.
233. **Riina Runnel.** Oral health among elementary school children and the effects of polyol candies on the prevention of dental caries. Tartu, 2015, 112 p.
234. **Made Laanpere.** Factors influencing women's sexual health and reproductive choices in Estonia. Tartu, 2015, 176 p.
235. **Andres Lust.** Water mediated solid state transformations of a polymorphic drug – effect on pharmaceutical product performance. Tartu, 2015, 134 p.
236. **Anna Klugman.** Functionality related characterization of pretreated wood lignin, cellulose and polyvinylpyrrolidone for pharmaceutical applications. Tartu, 2015, 156 p.
237. **Triin Laisk-Podar.** Genetic variation as a modulator of susceptibility to female infertility and a source for potential biomarkers. Tartu, 2015, 155 p.
238. **Mailis Tõnisson.** Clinical picture and biochemical changes in blood in children with acute alcohol intoxication. Tartu, 2015, 100 p.
239. **Kadri Tamme.** High volume haemodiafiltration in treatment of severe sepsis – impact on pharmacokinetics of antibiotics and inflammatory response. Tartu, 2015, 133 p.

240. **Kai Part.** Sexual health of young people in Estonia in a social context: the role of school-based sexuality education and youth-friendly counseling services. Tartu, 2015, 203 p.
241. **Urve Paaver.** New perspectives for the amorphization and physical stabilization of poorly water-soluble drugs and understanding their dissolution behavior. Tartu, 2015, 139 p.
242. **Aleksandr Peet.** Intrauterine and postnatal growth in children with HLA-conferred susceptibility to type 1 diabetes. Tartu. 2015, 146 p.
243. **Piret Mitt.** Healthcare-associated infections in Estonia – epidemiology and surveillance of bloodstream and surgical site infections. Tartu, 2015, 145 p.
244. **Merli Saare.** Molecular Profiling of Endometriotic Lesions and Endometria of Endometriosis Patients. Tartu, 2016, 129 p.
245. **Kaja-Triin Laisaar.** People living with HIV in Estonia: Engagement in medical care and methods of increasing adherence to antiretroviral therapy and safe sexual behavior. Tartu, 2016, 132 p.
246. **Eero Merilind.** Primary health care performance: impact of payment and practice-based characteristics. Tartu, 2016, 120 p.
247. **Jaanika Kärner.** Cytokine-specific autoantibodies in AIRE deficiency. Tartu, 2016, 182 p.