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IMPACT OF INFLAMMATION, OXIDATIVE STRESS AND AGE ON ARTERIAL STIFFNESS AND CAROTID ARTERY INTIMA-MEDIA THICKNESS

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To my family

"longevity is a vascular question. A man is old as old as his arteries"
Dr. William Osler, Principle and Practice of Medicine 1892

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LIST OF ORIGINAL PUBLICATIONS

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

I Kampus P, Kals J, Ristimäe T, Fischer K, Zilmer M, Teesalu R. High-sensitivity C-reactive protein affects central haemodynamics and augmentation index in apparently healthy persons. Journal of Hypertension 2004; 22:1133–1139.

II 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. International Journal of Cardiology 2006; 112:46–51.

III 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. Journal of Hypertension 2007; 25:819–825.

IV Kampus P, Kals J, Pihl E, Zilmer K, Pulges A, Teesalu R, Normak A, Zilmer M. Association between arterial elasticity, C-reactive protein and maximal oxygen consumption in well–trained cadets during three days extreme physical load: a pilot study (submitted for publication).

ABBREVIATIONS

AIx	augmentation index
AIx HR 75	augmentation index corrected for heart rate 75 beats *per* minute
BMI	body mass index
BP	blood pressure
C1	large artery elasticity index
C2	small artery elasticity index
CAD	coronary artery disease
CRP	C-reactive protein
CVD	cardiovascular disease
DBP	diastolic blood pressure
Hcy	homocysteine
HDL	high-density lipoprotein
ICAM-1	intercellular adhesion molecule- 1
IL-1	interleukin-1
IL-6	interleukin-6
IMT	carotid artery intima-media thickness
LDL	low-density lipoprotein
MAP	mean arterial pressure
NO	nitric oxide
OxLDL	oxidized low-density lipoprotein
PP	pulse pressure
PWA	pulse wave analysis
PWV	pulse wave velocity
RBC	red blood cell count
SBP	systolic blood pressure
TNF-α	tumor necrosis factor alpha
Tr	timing of the reflected waveform
VCAM-1	vascular cell adhesion molecule-1
VO_2max	maximal oxygen consumption
VO_2max/kg	maximal oxygen consumption *per* kilogram
WBC	white blood cell count

1. INTRODUCTION

Inflammation plays a key role in the pathogenesis of atherosclerosis, provides a patophysiologic link between early lesion formation and plaque rupture leading to occlusion and infarction (Ross 1999; Libby 2002). Development of atherosclerosis depends on a fragile balance between proinflammatory stimuli on one hand and anti-inflammatory and antioxidative defense mechanism on the other hand. The imbalance of this mechanism leads to endothelial dysfunction and accumulation of oxidized low-density lipoprotein (oxLDL) within arterial wall, contributing to the inflammatory state of atherosclerosis (Ross 1999). The inflammatory process in the atherosclerotic artery leads to increased blood levels of inflammatory cytokines and other acute-phase reactants. Epidemiological and clinical studies have shown strong and consistent relationship between the markers of inflammation and the risk for future cardiovascular disease (CVD) (Blake *et al*. 2001). To date, C-reactive protein (CRP) is the most promising of these biomarkers for prediction of cardiovascular risk in terms of clinical utility (Blake *et al*. 2001; Pearson *et al*. 2003; Willerson *et al*. 2004; Kushner *et al*. 2006).

Oxidized LDL is a pro-inflammatory and pro-atherogenic lipoprotein which is also intimately involved in initiation, progression and potentially in destabilization of atherosclerotic lesions (Tsimikas *et al.* 2004a). OxLDL results from exposure of LDL to oxidizing species. Levels of oxLDL in the plasma and in plaques correlate with and colonize with macrophage infiltration within plaques (Nishi *et al.* 2002). Plasma levels of oxLDL are independently associated with the risk of CVD (Shimada *et al.* 2004). Homocysteine (Hcy) has also been suggested to be an independent risk factor for CVD (Clarke *et al.* 1998). However, the mechanisms through which elevated Hcy promotes atherosclerotic disease are not completely understood. Laboratory studies have suggested that hyperHcy may also induce the atherosclerotic process via high-grade oxidative stress (Virdis *et al.* 2001).

Functional and structural changes of the arteries are important part of vascular ageing, become precursors of premature CVD and powerful predictors of cardiovascular events (Laurent *et al*. 2006). Noninvasive identification of such alterations in arterial function and structure provides a means for early detection of presymptomatic vascular disease. Recently, new computerized equipments and non-invasive techniques of pulse wave analysis (PWA) by applanation tonometry have been developed, which provide a simple reproducible methods to assess the indices of arterial stiffness and central blood pressure (BP) (Nichols and O'Rourke 1998; Glasser *et al.* 1997). Due to the heterogeneity of arterial stiffness through the arterial tree, with more elastic proximal arteries and stiffer distal arteries, pulse pressure (PP), measured conventionally from the brachial artery, does not reflect actual central PP. In young adults the amplitude of brachial pressure may be 50% greater than the pressure in ascending aorta,

while in the elderly it becomes almost equal (Wilkinson *et al*. 2000a; Hirata *et al*. 2006). Therefore, estimation of arterial stiffness and central BP by noninvasive PWA has become an important part of cardiovascular risk stratification.

High resolution B-mode ultrasound is a noninvasive method for examining the thickness of arterial wall and provides a measure of carotid artery intima-media thickness (IMT). Increased IMT is predictive of cardiovascular events in asymptomatic individuals (Chambless *et al.* 1997; Rosvall *et al.* 2005). IMT has been used in research settings to identify patients with subclinical atherosclerosis, and is considered to be a validated surrogate marker of systemic atherosclerotic disease (Salonen and Salonen 1993).

The status of inflammatory and oxidative stress has been suggested to be associated with the functional and structural properties of the arterial wall. CRP has been shown to be associated with endothelial function (Fichtlscherer *et al*. 2000) and with increased peripheral PP (Abramson *et al.* 2002a), as a surrogate measure of arterial stiffness. Previous studies have demonstrated association between arterial stiffness and endothelial function (Ravikumar *et al*. 2002), which allows to suggest that inflammation may be also involved in arterial stiffening. There is no data on association between oxidative stress and arterial stiffness. The results published in different studies about associations of novel cardiovascular risk factors, e.g. CRP, oxLDL and, Hcy with IMT in healthy persons are also discordant.

The main purpose of the present study was to investigate the impact of age, inflammatory and oxidative stress related markers on arterial stiffness and IMT in different clinical settings.

2. REVIEW OF THE LITERATURE

2.1. Inflammation in the pathogenesis of cardiovascular disease

Inflammation plays a key role in the pathogenesis of atherosclerosis, provides a patophysiologic link between early lesion formation and plaque rupture, leading to occlusion and infarction (Ross 1999; Libby 2002). The first step in the development of an atheromatous lesion is endothelial dysfunction, which is caused by several risk factors, e.g. elevated oxLDL, presence of free radicals caused by cigarette smoking, diabetes, hypertension, obesity, etc. Damaged endothelial cells express adhesion molecules including selectins, P- and E-selectin and members of immunoglobulin superfamily, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (Kasper *et al*. 1996). Selectines mediate transient rolling of the leucocytes along the endothelium, while stronger attachment is mediated by ICAM-1 and VCAM-1. Leukocytes, especially T-lymphocytes and monocytes, are then migrating into the subendothelial space, attracted by monocyte chemoattractant protein-1 (Gu *et al*. 1998). Local inflammatory response, with the macrophage colony stimulating factor combining monocyte chemoattractant protein-1, stimulates transforming of monocytes into the macrophages. Particles of LDL extravagate through the "leaky" defective endothelium into the subendothelial space, where atherogenetic lipoproteins are retained and modified. The macrophage binds and takes up modified LDL and converts the macrophage into the foam cell (Qiao *et al*. 1997). Macrophages also secrete a number of cytokines, e.g. tumour necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), thus creating a cycle where more foam cells are generated. At the same time, T-lymphocytes lead to expression of interferon-γ and lymphotoxin, which stimulate the pro-inflammatory state and promote migration and proliferation of smooth muscle cells. Macrophages, endothelial cells and smooth muscle cells produce TNF-α, which together with interferon-γ and IL-1, stimulates production of interleukin-6 (IL-6). IL-6 is the main hepatic stimulus for the acute phase reactant CRP.

Accumulation of foam cells leads to formation of the lipid core. The fibrous cap separates the lipid pool from the luminal blood. The mechanical strength and stability of the fibrous cap is provided by collagen, which is produced by smooth muscle cells. Synthesis and breakdown of collagen is controlled by inflammatory signals (Libby 1995). It has been demonstrated that the platelet-derived growth factor and the transforming growth factor-β increase the production of collagen by smooth muscle cells, whereas interferon-γ inhibits collagen synthesis, activating macrophages to secrete matrix metalloproteinases that proteolytically degrade collagen, promoting the fibrous cap to rupture (Ross

1999; Libby 2002). Thus, inflammation plays a central role in the initiation and progression of atherosclerosis as well as in the thrombotic complications of this disease.

2.1.1. C-reactive protein

The inflammatory process in the atherosclerotic artery leads to increased blood levels of inflammatory cytokines and other acute-phase reactants. Epidemiological and clinical studies have shown strong and consistent relationship between the markers of inflammation and risk for cardiovascular events (Blake *et al.* 2001). To date, CRP is the most promising of these biomarkers for prediction of cardiovascular risk in terms of clinical utility (Blake *et al.* 2001; Pearson *et al.* 2003; Willerson *et al.* 2004; Kushner *et al.* 2006).

C-reactive protein was discovered in 1930 by Tillet and Francis, when they studied the immune response of patients with pneumococcal pneumonia. The investigators found that the sera of these patients precipitated with the soluble extract of *pneumococcus pneumonia*e and the extract was called fraction C. In 1941, Avery and Abernethy discovered that this reactive substance was protein - C-reactive protein (Ablij *et al.* 2002).

C-reactive protein is an acute-phase reactant that is mainly synthesized by hepatocytes in response to acute injury, infection or other inflammatory stimuli. The main stimuli for secretion of CRP are IL-1, IL-6 and indirectly TNF-α. The human CRP gene is located on the long arm of chromosome 1. CRP is a pentameric protein consisting of five noncovalently bound identical subunits with an overall weight of approximately 11800 daltons (Ablij *et al.* 2002). Small amounts of CRP can also be produced locally. CRP has been detected on the surface of about 4% of normal blood lymphocytes (Kuta and Baum 1986). Moreover, in atherosclerotic lesion, smooth muscle cells (Calabro *et al.* 2003; Jabs *et al.* 2003) and monocytic cells (Yasojima *et al.* 2001) are able to produce CRP.

Activation of the classical pathways of the complement system and the ability to modulate the function of phagocytic cells represent the direct biological function of CRP. CRP undergoes calcium-dependent binding to choline phosphates, polysaccharides and peptopolysaccharides present on bacteria, parasites, and fungi. CRP plays a role in opsonization of infectious agents and damaged cells (Ballou and Kushner 1992).

Normally, CRP is present in traces in the serum, but increases rapidly up to 300 mg in response to a variety of infections or inflammation (Gabay and Kushner 1999). In healthy young adults the median concentration of CRP is 0.8 mg/l, the 90^{th} centile is 3.0 mg/l and the 99^{th} centile is 10 mg/l (Shine *et al.* 1981). After a single stimulus, predominantly under the transcriptional control of IL-6, hepatic synthesis starts very rapidly rising above 5 mg/l in about 6

hours and peaking in about 48 hours (Pepys and Hirschfield 2003). Due to its pentraxin structure, the plasma half-time of CRP is about 19 hours and the only determinant of circulating CRP concentration is the rate of its synthesis (Vigushin *et al.* 1993), reflecting the intensity of the pathological process. Studies have shown that CRP concentration during the day and over days to a month and even over months to a year is relatively constant in an individual, which makes it possible to measure CRP at any time of the day (de Ferranti and Rifai 2002). CRP concentration tends to increase minimally with age and to differ between different ethnic groups, as well as between males and females (Imhof *et al.* 2003; Ford *et al.* 2003; Rifai *et al.* 2003).

Thirty years ago CRP was measured using qualitative or semi-quantitative laboratory technique (e.g. latex agglutination). Today, accurate and rapid quantitative measures of CRP are used. High-sensitivity methods with lower detection limits of less than 0.1 mg/l allow differentiation between the low-level states of inflammation (subclinical inflammation) that are important in assessment of cardiovascular risk (Pearson *et al.* 2003).

C-reactive protein and cardiovascular risk

C-reactive protein is the most frequently studied inflammatory marker in epidemiological research. In healthy women and men CRP predicts future cardiovascular risk independently of traditional cardiovascular risk factors (Ridker *et al*. 1997; Ridker *et al*. 1998). Today, more than 20 prospective studies have shown a consistent and robust relationship between CRP, measured by high-sensitivity assays, and the risk of future cardiovascular events (Scirica and Morrow 2006). Compared with the upper and lower tertiles of CRP, the relative risk for major cardiovascular events increases 2-fold independently of classical risk factors (Scirica and Morrow 2006). Moreover, analyses of population based data in the Women's Health Study indicate that CRP is a stronger predictor of future cardiovascular events than LDL cholesterol and hence provide additional prognostic information in patients with low LDL cholesterol level (Ridker *et al.* 2002). The American Heart Association categorised CRP values, based on cardiovascular risk, into three groups, low (CRP <1 mg/l), mild (CRP 1–3 mg/l) and high (CRP >3 mg/l), and suggested their high utility in screening, particularly for those at intermediate risk according to cardiovascular risk estimation algorithms (Pearson *et al.* 2003).

Risk factor modification techniques such us increased physical activity, moderate alcohol intake, weight loss, and smoking cessation lowers CRP. Of the pharmacological agents, statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) therapy has been shown to provide significant reduction of CRP levels independently of blood lipid change (Ridker *et al.* 1999; Jialal *et al.* 2001; Chan *et al.* 2004). The ability of statin therapy to reduce CRP levels is clearly a class effect, which is not mediated by LDL cholesterol reduction (Willerson and Ridker 2004). Moreover, in primary prevention study, statin

treatment was effective in preventing acute coronary events among those with elevated CRP levels, regardless of the baseline level of LDL cholesterol (Ridker *et al.* 2001). However, there has been no evidence of the fact that the reduction of cardiovascular events is due to CRP reduction *per se* and that statin therapy should be recommended in persons with no prior history of CVD on the basis of elevated CRP alone in primary prevention.

C-reactive protein as a mediator of cardiovascular disease

In recent years, there has been accumulated increasing evidence of the direct atherogenetic mechanisms of human CRP (Li *et al.* 2004). In animal studies human CRP increases the size of cerebral and myocardial infarction (Gill *et al.* 2004). In laboratory studies CRP enhanced expression of local endothelial cell surface adhesion molecules (Pasceri *et al.* 2000), monocyte chemoattractant protein-1 (Pasceri *et al.* 2000; Pasceri *et al.* 2001), endothelin-1 (Verma *et al.* 2002a) and endothelial plasminogen activator inhibitor-1 (Devaraj *et al.* 2003). Native CRP binds to oxLDL, degrades partly and then activates the complement (Pepys and Hirschfield 2003). CRP reduces directly the bioactivity of endothelial nitric oxide (NO) (Verma *et al.* 2002b; Venugopal *et al.* 2002; Clapp *et al.* 2005), inhibits directly differentiation, survival, and function of endothelial progenitor cells (Verma *et al.* 2004), increases induction of the tissue factor in monocytes (Nakagomi *et al.* 2000), LDL uptake by macrophages (Zwaka *et al.* 2001) and colocalization with the complement membrane attack complex within atherosclerotic lesions (Torzewski *et al.* 1998). In addition, evidence suggests that CRP and complement proteins are also synthesized within atherosclerotic lesions. The CRP mRNA plaque levels were found to be 10.2-fold higher than the corresponding levels in the normal artery and 7.2-fold higher than in the liver (Yasojima *et al.* 2001).

2.2. Oxidative stress

Development of atherosclerosis depends on a fragile balance between pro-inflammatory stimuli on one hand and on the anti-inflammatory and anti-oxidative defense mechanism on the other hand (Ross 1999). Oxidative stress is defined as an imbalance between pro-oxidants (reactive oxygen and nitrogen species) and antioxidants in favor of pro-oxidants (Zilmer *et al.* 1999). Formation of reactive species is a part of the nonspecific defense system of the human body. However, chronic and acute overproduction of reactive species under pathophysiological conditions is relevant for development of CVD (Madamanchi *et al.* 2005). Determination of oxidative stress is mainly based on determination of the products of lipid peroxidation and oxidatively modified compounds.

2.2.1. Oxidized low-density lipoprotein

Oxidative modification of LDL in atherosclerotic lesions was demonstrated conclusively in the 1980s, and various oxidation products have since been documented to be present in the diseased arterial wall (Davies *et al.* 1999). One step in the pathogenesis of atherosclerosis is an accumulation of oxLDL within plaques, which contributes to the inflammatory state, and plays an important role in the pathogenesis of atherosclerosis (Ross 1999). OxLDL results from exposure of LDL to oxidizing species. This modification renders it unable to be recognized by LDL receptors and oxLDL is taken up by a variety of scavenger receptors present on macrophages in the artery wall, resulting in accumulation of cholesterol esters and generation of foam cells (Zilmer *et al.* 1999). OxLDL has a toxic effect on endothelial cells and also on monocytes and T-cells, especially at higher concentrations (Colles *et al.* 2001). Incubation of endothelial cells with oxLDL stimulated O_2^- formation (Cominacini *et al.* 1998). Moreover, animal experiments have demonstrated that immunization of animals with oxLDL leads to enhancement of autoantibodies to oxLDL, which inhibits development of atherosclerosis (Freigang *et al.* 1998).

Direct measurement/visualization of oxLDL in the vessel wall is an area of active study (Torzewski *et al.* 2004), but is not currently available in humans. Development of monoclonal antibodies binding oxidation-specific epitopes has allowed development of sensitive and specific assays for measurement of circulating plasma oxLDL (Holvoet *et al.* 1995; Itabe *et al.* 1996). The relationship of oxLDL with cardiovascular risk factors is not fully established, because most of oxLDL is present in the vessel wall rather than in the plasma (with up to 100-fold differences). Studies have shown that levels of oxLDL in the plasma and in plaques correlate with and colonize with macrophage infiltration within plaques (Nishi *et al.* 2002).

Oxidized LDL levels have been associated with LDL cholesterol (Holvoet *et al.* 2003) but also with inflammatory markers, adhesion molecules, cytokines and CRP (Hulthe and Fagerberg 2002). OxLDL levels in the plasma correlated with plaque progression (Hulthe and Fagerberg 2002; Wallenfeldt *et al.* 2004) and associated with IMT (Liu *et al.* 2004). OxLDL is increased in hypertensive patients (Frostegård *et al.* 2003). In a recent study evaluating a population undergoing coronary angiography, oxLDL levels correlated with both the presence and extent of CAD (Tsimikas *et al.* 2005). The association between oxLDL and CAD has been confirmed also in previous studies (Holvoet *et al.* 2001; Shimada *et al.* 2004; Tsimikas *et al.* 2004b). In the Bruneck study (Tsimikas *et al.* 2006) oxLDL levels were significantly associated with the presence, extent, and development of carotid atherosclerosis. However, there were not enough new cardiovascular events at the end of the study to determine the predictive value of these events. In another study oxLDL was associated with future CVD in apparently healthy men with the moderate absolute risk of

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CVD. OxLDL was even a stronger predictor of cardiovascular risk than standard lipid variables and other traditional cardiovascular risk factors (Meisinger *et al.* 2005). A recent meta-analysis has clearly shown that oxLDL is related to increased cardiovascular risk in healthy persons (Lobbes *et al.* 2006). OxLDL has also been studied as a predictor of secondary cardiovascular events. In patients with CAD, baseline levels of oxLDL were independent predictors of cardiac death, nonfatal myocardial infarction and unstable angina (Shimada *et al.* 2004).

The impact of statin therapy and diet on oxLDL is currently an issue of active research. Cross-sectional and prospective studies have demonstrated that oxLDL is lower in patients using statins, or decrease in patients treated with a variety of statins (Van Tits *et al.* 2006; Diepeveen *et al.* 2005). In hypertensive patients treatment with both candesartan and amlodipine was associated with the decrease in oxLDL, whereas no impact was seen on serum lipid levels (Muda *et al.* 2006).

2.2.2. Oxidative stress and homocysteine

Homocysteine is an sulfur-containing amino acid, generated during the metabolism of methionine. Hcy is remethylated to methionine or is broken down into cysteine and gluthathione. Those processes are catalyzed by 2 main enzymes: cystathione-ß-synthase and methionine synthase and require vitamins B_6, B_{12} and folic acid as cofactors, which deficiency is a frequent cause of hyperHcy (Stanger *et al.* 2003). Hcy has also been suggested to be an independent risk factor for CVD (Clarke *et al.* 1998). The mechanisms by which elevated Hcy promotes atherosclerotic disease are not completely understood. Laboratory investigations have revealed several potential mechanisms, including impairment of endothelial function (Woo *et al.* 1997), oxidation of LDL (Parthasarathy 1987), increased monocyte adhesion to vessel wall (Welch and Loscalzo 1998), increase lipid uptake and retention (Welch and Loscalzo 1998), activation of the inflammatory pathway (Hofmann *et al.* 2001) and stimulatory effects on smooth-muscle proliferation (Welch and Loscalzo 1998). Voutilainen *et al.* (1999) demonstrated that plasma Hcy is associated with enhanced systemic lipid peroxidation. According to previous study there is more evidence that hyperHcy may induce atherosclerotic process via high-grade oxidative stress (Virdis *et al.* 2001). Moreover, in humans, hyperHcy impairs endothelium-dependent vasodilatation in the brachial artery, which is prevented by administration of C-vitamin (Chambers *et al.* 1999; Kanani *et al.* 1999). On the contrary, a recent study demonstrated that lowering plasma Hcy through folate supplementation was not associated with any change in the measures of antioxidative activity or oxidant damage (Moat *et al.* 2003). Moreover,

causative evidence for high Hcy levels, leading to atherosclerosis, has been questioned in the last few years (Darius *et al.* 2003; Kaul *et al.* 2006).

2.3. Arterial stiffness

During the last decade great progress has been made in recognizing the role of arterial stiffness in the pathogenesis of CVD. Increasing arterial stiffness is the hallmark of the ageing process and has emergences as an important determinant of increased systolic blood pressure (SBP) and PP and an important cardiovascular risk factor (Laurent *et al.* 2006).

The mechanism underlying age-dependent development of vascular stiffness is largely unknown, but it is known to be independent of mean arterial pressure (MAP) or other presence risk factors (Benetos *et al.* 2002). Compared to age-dependent changes of the central elastic arteries, there are only small alterations of the peripheral muscular arteries with age.

The composition and structure of the extracellular matrix and cell-matrix interactions are the most important determinants of arterial stiffness. However, the signaling cascades involved in these processes are not fully understood. It has been suggested that the components of the renin-angiotensis system, endothelial alterations, intracellular signaling, components of the extracellular matrix and matrix metalloproteinases may be involved in the cascade of events that results in arterial stiffening (Intengan and Schiffrin 2001; Schiffrin 2004).

The basic morphological pattern of the large arteries consists of the cells and the matrix arranged in three transmural zones: the intima, the media, and the adventitia. At its luminal side the intima consists of a single continues layer of endothelial cells which is a rich source of substances and signal transduction mechanisms that influence the mechanical properties of the whole vessel wall. Endothelial cells continuously synthesize NO and endothelin-1, which are both involved in regulation of arterial stiffness (Wilkinson *et al.* 2002a; McEniery *et al.* 2003).

The internal elastic lamina separates the intima from the media. The media layer represents the main basis of the mechanical properties of the elastic arteries. It is composed of a number of layers of elastic lamellae, smooth muscle cells and the connecting molecular gird. The smooth muscle cells consist of different mixtures of phenotypes, which determine its contractile, proliferative, synthetic or apoptotic behavior, influenced by age, location in the vascular tree, and pathological condition (Van Bortel *et al.* 2001).

The third layer is the adventitia, which is abundant in the more centrally located large arteries and consists mainly of fibroblasts and collagen. Collagen is a major determinant of the stiffness of the large arteries. Collagen is approximately 500 times stiffer than elastin ant it more than doubles in content from age 20 to 70 years (Nichols and O'Rourke 1998). During lifetime, colla-

gen is subjected to chemical modifications, such us breakdown, cross-linking, and glycation, which have a profound influence on the mechanical properties of the large arteries (Van Bortel *et al.* 2001). Collagen and elastin provide structural integrity and elasticity, and are potently regulated by catabolic matrix metalloproteases. Moreover, recent data indicate a possible link between elastases, including matrix metalloproteinase, in the process of arterial stiffening (Yasmin *et al.* 2005). Inflammatory cells are also involved in the production of collagenases and elastases (Jacob 2003), but whether inflammation leads to arterial stiffening is still unclear.

Hypertension and arterial stiffness

Ageing and hypertension are the two main conditions, which mainly increase the stiffness of the central arteries compared to the peripheral medium-sized arteries.

Hypertension is a common and powerful modifiable risk factor for CVD. Hypertension has been defined as SBP ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg. In the hypertensive subjects CVD sequels occur at 2- to 4- fold increased rate in comparison with normotensive subjects. The risk of CVD increases with each increment in BP, even with the high-normal range (Kannel and Wilson 2003). Based on a metaanalysis of long-term clinical trials, the rates of CVD events for treated hypertensive individuals with BP below the 120/80 mmHg were twofold lower than for those with BP above 140/90 mmHg (Lewington *et al.* 2002).

The etiology of essential hypertension has not been fully elucidated mostly because of as yet unknown genetic variation and multiple nonhereditary factors that have important and modifiable influences on BP (Carretero and Oparil 2000). Hypertension tends to occur in association with other atherogenetic risk factors that promote its occurrence and strongly influence its CVD impact. Hypertension appears to be metabolically linked to dyslipidemia, glycose intolerance, abdominal obesity, hyperinsulinemia and hyperuricemia (Kannel and Wilson 2003). Isolated systolic hypertension is thought to result in part from age-associated vascular stiffening and reduced compliance and distensibility of the central conduit arteries.

In hypertension the principal structural modification of vessel wall is hypertrophy of the media layer and excessive collagen production (Laurent *et al.* 1994; Xu *et al.* 2000). The diameter of the central arteries increases proportionately with the level of BP, and medial hypertrophy involves a marked increase in the extracellular matrix and adventitia, which leads to increased stiffness of the large arteries (Blacher *et al.* 1999a). Studies have demonstrated increased arterial stiffness in patients with essential hypertension (Williams *et al.* 2006; Cohn *et al.* 1995; Glasser *et al.* 1997; McVeigh *et al.* 1999). Moreover, indices of arterial stiffness have an independent predictive value for all-cause and cardiovascular mortalities, fatal and nonfatal coronary events, and

fatal strokes in patients with essential hypertension (Laurent *et al.* 2001; Boutouyrie *et al.* 2002; Laurent *et al.* 2003).

During past years an increasing body of evidence has suggested that inflammation plays an important role in development of essential hypertension. Cross-sectional studies have demonstrated higher CRP levels among individuals with elevated BP (Bautista *et al.* 2001; Chae *et al.* 2001). Moreover, the data from the Women's Health Study provides evidence that baseline CRP are modestly but independently associated with increased incidence of hypertension, even in patients with very low initial BP (Sesso *et al.* 2003; Sesso *et al.* 2007). It has been suggested that higher levels of CRP may increase BP by up-regulating angiotensin type 1 receptor expression (Wang *et al.* 2003) and by reducing NO production in endothelial cells (Verma *et al.* 2002b; Venugopal *et al.* 2002), resulting in vasoconstriction and increased production of endothelin 1 (Verma *et al.* 2002a; Devaraj *et al.* 2003). Recently, there has been suggested a link between inflammation and peripheral PP, as a surrogate measure of arterial stiffness in hypertensive patients (Schillaci *et al.* 2003).

2.3.1. Methodologies for assessment of arterial stiffness

Invasive and noninvasive methods have been introduced for assessment of arterial stiffness. Invasive methods provide data of arterial blood flow, pressure, and diameter change but are not used routinely in clinical practice. These methods are mostly used in laboratories for animal experiments, providing a basis for measurement and interpretation of non-invasive data in clinical settings. Noninvasive measurement of arterial stiffness involves three main methods: 1) pulse travel time, 2) analysis of arterial pressure pulse and its wave contour, and 3) direct estimation of stiffness using measurements of diameter and distending pressure (Pannier *et al.* 2002). A number of computerized devices are now available that enable quantification of the systemic indices of stiffness, regional and local measurements of arterial stiffness and wave reflection. Systemic arterial stiffness can be estimated only on the basis of the model of the circulation, whereas wave reflection, regional and local arterial stiffness can be measured directly and noninvasively at various sites along the arterial three (Laurent *et al.* 2006).

Pulse wave velocity (PWV), determining regional arterial stiffness from the aorta, is generally accepted as the "gold standard" for measurement of arterial stiffness (Laurent *et al.* 2006). PWV is a simple, non-invasive, reproducible method for assessment of arterial stiffness; it is calculated by measuring the time taken by the arterial waveform to pass between two points (usually between the carotid artery and the femoral artery) (Mackenzie *et al.* 2002). Moreover, PWV is an independent predictor of outcome in a variety of populations (Blacher *et al.* 1999b; Lauren *et al.* 2001; Willum-Hansen *et al.* 2006).

Local arterial stiffness can be determined from the superficial arteries, mainly the brachial, femoral and carotid arteries, using ultrasound devices, for measurement of stroke-caused changes in the diameter and local PP. Ultrasound based methods require good technical experience and longer time to detect very small changes in vessel diameter, which makes it difficult to use in large epidemiological studies (Mackenzie *et al.* 2002; Laurent *et al.* 2006).

2.3.2. Pulse wave analysis

2.3.2.1. Historical review

Assessment of arterial pulse as a diagnostic tool has been an important part of clinical examination for centuries. Many centuries before Christ, the Egyptians described the pulse wave as the "word" of the heart to the vessels. Later on, the Chinese started to analysed the pulse and the pulse amplitude was used as an index of vivacity, vigour and health (Asmar 1999). Today, use of pulse diagnostics is still a part of traditional Chinese medicine. In 130 –200 ^{AD} Calen palpated pulse and classified it in terms of strength, rate and rhythm (Nichols and O'Rourke 1998). The first sphygmograph, "the pulse writer", was introduced in the early 1860s by the Parisian physiologist Marey. Some years later Mahomed (1872) and Mackenzie (1902) from London developed various types of sphygmographs and started to use them in clinical practices. In the late 1870s Mahomed described changes in arterial pulse in patients with Bright disease (now thought to be essential hypertension) (Moss 2006). However, the developed sphygmographs were difficult to use in daily clinical practice. Soon the sphygmographs were replaced by the cuff sphygmomanometer introduced by Riva-Rocci in 1896. In 1905 the Korotkov auscultatory method for recording SBP and DBP replaced the Riva-Rocci palpatory method and clinicians began to use sphygmomanometers (Nabokov and Nevorotin 1998), while sphygmographs were buried in oblivion for a long time. Sphygmographs permitting numerical expression of the arterial waveform were not available until the late 20th century when the studies of McDonald and Taylor (1959) and Nichols and O'Rourke (1990) rediscovered the importance of the arterial waveform (Nichols and O'Rourke 1998). Owing to the introduction of catheter-tip manometers by Murgo and Millar and practical high-fidelity applanation tonometers (Kelly *et al.* 1989; O'Rourke and Gallagher 1996), O'Rourke and colleagues developed the technique of PWA, making noninvasive derivation of central pressure waveforms possible (O'Rourke and Gallanger 1996). The recent development of noninvasive methods such a ultrasound, pressure-sensitive transducer, applanation tonometry and photoplethysmography for recording arterial flow or pressure waves has opened up a new chapter. Noninvasive assessment of arterial function in epidemiological and therapeutic studies has

been firmly established, and has become an important tool as a therapeutic target as well as a basis in assessment of cardiovascular risk.

2.3.2.2. Principles of pulse wave analysis

The oldest Windkessel model of the circulation is a central elastic reservoir (the large arteries), into which the heart pumps, and from which blood travels to the tissues through the relatively nonelastic conduits (peripheral arteries). This model separates the "conduit" and the "cushioning" functions of the arterial tree (Nichols and O'Rourke 1998). The Windkessel model has two major limitations. First, the arterial tree does not have a separate conduit and cushioning functions. There occurs progressive loss of the cushioning function, from the ascending aorta to the more muscular and less elastic peripheral arteries, and the increasingly predominant conduit function of the large arteries from the heart to the periphery. Secondly, the Windkessel model is based on the assumption that PWV has an infinite value (Laurent *et al.* 2006).

The propagative models of the circulatory system assume that the velocity with which a pulse wave travels along a given artery has a finite value. The model consists of a simple distensible tube which terminates at the peripheral resistance, but whose distributed elastic properties permit the generation of a pressure wave witch travels along the tube (Nichols and O'Rourke 1998). The wave generated by cardiac activity travels through the tube to the periphery and is reflected back from the periphery. The pressure wave at any point along the tube is the result of the incident and of the reflected wave. When the tube is distensible the wave velocity is slow, and the reflected wave returns to the heart late, during the diastole. In the case of stiffened vessels the wave travels fast and the reflected wave merges with the systolic part of the incident wave. In the human body, wave reflections originate from various locations including peripheral bifurcations of the conducting arteries and the smaller muscular arteries. PWV increases progressively from the ascending aorta to the femoral arteries (4–5 m/s to 8–9 m/s) (Nichols and O'Rourke 1998). The heterogeneity of PWV along the arterial tree has an important physiological and pathophysiological consequence. As the peripheral arteries are stiffer than the central arteries, this phenomenon leads to an increase in the amplitude of the pressure wave in the vessels, from the heart to periphery, which is known as pressure amplification (amplification phenomenon).

2.3.2.3. Systolic pulse wave analysis

Peripheral PP, i.e. the difference between peripheral SBP and DBP, has been used as an indirect measure of arterial stiffness. SBP and DBP tend to increase with age, while at age 50–60 there is no further increase of DBP, it actually declines and the range of PP widens with age (Franklin *et al.* 1997). However, PP measured from the brachial artery does not actually reflect central PP due to

the “amplification phenomenon”. In young adults the amplitude of brachial pressure may be 50% greater than pressure in the ascending aorta, while it becomes almost equal in the elderly (Wilkinson *et al.* 2000a; Hirata *et al.* 2006). Central PP, but not brachial PP, represents the actually workload for the heart and is therefore a better predictor of left ventricular mass (Saba *et al.* 1993; Hashimoto *et al.* 2007) and cardiovascular risk (Safar *et al.* 2002).

Based on the wave propagative model of the arterial three, there has been developed a commercially available device Sphygmocor Px (AtCor, Sydney, Australia). Using high-fidelity applanation tonometry to record the peripheral pressure wave from the radial artery and applying the generalized transfer function (Chen *et al.* 1997), the Sphygmocor has possibilities for estimating the systolic pulse wave at the central level, i.e. in the ascending aorta. Karamanoglu *et al.* (1993) showed that generalized transfer function estimated central SBP, in comparison with invasively measured SBP, with a difference of 2.4±1.0 mmHg. Generalized transfer function has been validated by many studies (Chen *et al.* 1997; Pauca *et al.* 2001; Adji and O’Rourke 2004) and approved in the expert consensus document on arterial stiffness (Laurent *et al*. 2006).

Augmentation index and the timing of the reflected waveform

The arterial pressure waveform at any point of the arterial tree is a composite of the forward pressure wave, generated by ventricular contraction, and the reflected wave. In elastic vessels the reflected wave tends to return to the aortic root during the diastole. In stiffer arteries the reflected wave arrives to the aortic root earlier, thus adding to the forward wave and augmenting systolic pressure. The forward and backward travel waves can be quantified through the augmentation index (AIx)–defined as the difference between the second (P2) and the first (P1) systolic peaks, expressed as the percentage of PP (Figure 1), which has been used as indirect surrogate measure of arterial stiffness (Laurent *et al.* 2006). A number of studies have demonstrated the good reproducibility of AIx in different clinical settings (Wilkinson *et al.* 1998; Siebenhofer *et al.* 1999). For example, AIx showed 0.23±0.66% of inter-observer difference and 0.49±0.93% of intra-observer difference in healthy subjects (Wilkinson *et al.* 1998).

Systolic PWA provides also a measure of the timing of the reflected waveform (Tr) (Figure 1). Tr is the composite travel time of the pulse wave to the periphery, the main reflectance site (aortic bifurcation) and its return to the ascending aorta. Tr correlates strongly with carotid-femoral PWV (London *et al.* 1992) and has been used as the estimated measure of aortic stiffness (e.g carotid-femoral PWV) (Marchais *et al.* 1993; Wilkinson *et al.* 2001a; Wilkinson *et al.* 2002b). However, these parameters are not absolutely identical, Tr is responsive, at least in part, to wave reflection, while carotid–femoral PWV characterizes more directly pulse wave transmission in the aorta.

The reflected wave depends on several factors including, the elastic properties of the elastic and muscular arteries, on PWV, on the timing of the reflected wave and the distance of the major reflecting sites (Nichols and O'Rourke 1998). According to previous studies (Cameron *et al.* 1998; Hayward and Kelly 1997) it is well established that AIx is influenced by age. Age is the main determinant of arterial stiffness. Recently, McEniery demonstrated that changes in AIx were more prominent in younger individuals (<50 years), whereas changes in PWV were more marked in older individuals, suggesting that AIx might be a more sensitive marker of arterial ageing in younger individuals (McEniery *et al.* 2005). Gender is also an important determinant of AIx (Hayward and Kelly 1997; Cameron *et al.* 1998). The higher AIx in female subjects is probably due to shorter height and hence to closer proximity between the heart and the sites of reflection. Heart rate and height are inversely related to AIx. Wilkinson *et al.* (2000a; 2002c) demonstrated that increasing heart rate using right atrial pacing was accompanied by a linear decrease of 3.8% to 5.6% *per* each 10 beats/min in AIx. As the shorter distance for the waveform leads to the quicker return of the reflected wave (Smulyan *et al.* 1998), smaller height is accompanied with increase of AIx. AIx also depends on MAP. It has been demonstrated that infusion of angiotensin II and noradrenalin resulted in a significant increase of AIx and decrease of Tr to a similar degree, for a given increment in MAP (Wilkinson *et al.* 2001a).

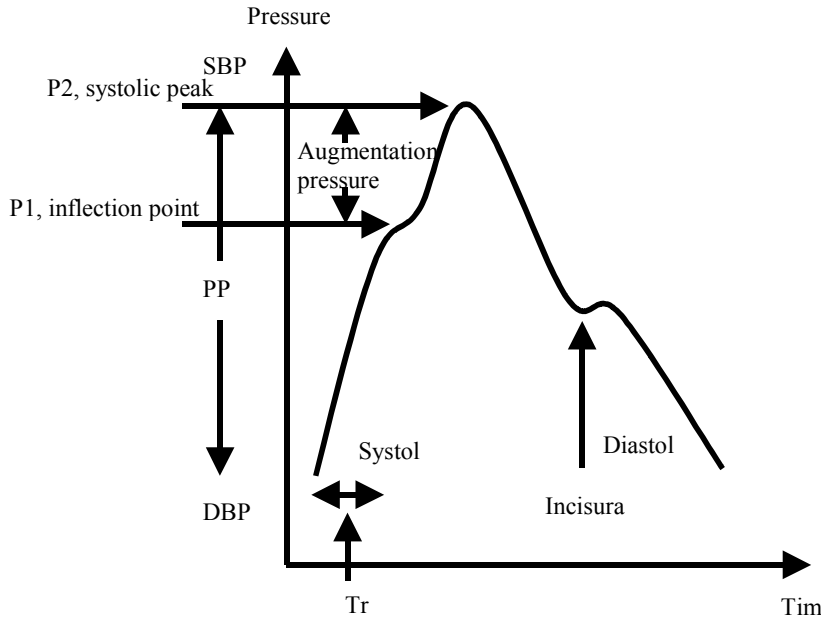

Figure 1. Central arterial pressure waveform. AIx is calculated as the difference between P2 and P1, expressed as percentage of the PP. Tr is defined as the time between the foot of the wave and the inflection point. Modified from (Kals *et al.* 2003).

Age, gender, heart rate, height and MAP have been critically analysed in the literature and it is generally accepted that all the above mentioned determinants should be considered in the analysis of the indices of systolic PWA (Davies and Struthers 2003; Oliver and Webb 2003).

Wave reflection and cardiovascular risk

Many observation studies have reported various patohysiological conditions which are associated with increased wave reflection. AIx increased in patients with diabetes mellitus (Wilkinson *et al.* 2000b), hypercholesterolemia (Wilkinson *et al.* 2002b), essential hypertension (Williams *et al.* 2006), CAD (Weber *et al.* 2005), peripheral arterial disease (Kals *et al.* 2006a), end-stage renal disease (London *et al.* 2001), rheumatoid arthritis (Klocke *et al.* 2003), systemic vasculitis (Booth *et al.* 2004) and obesity (Shargorodsky *et al.* 2006). Central PP and AIx are associated with left ventricular hypertrophy (Saba *et al.* 1993; London *et al.* 1994; Hashimoto *et al.* 2007), which is a known risk factor for coronary events in normotensive and hypertensive patients (Westerhof and O'Rourke 1995; Levy *et al.* 1990), microalbuminuria (Tsioufis *et al.* 2003) and endothelial function (Ravikumar *et al.* 2002; Kals *et al.* 2006a). Moreover, a link has also been demonstrated between AIx and low birth weight (Lurbe *et al.* 2003), which is considered as a risk factor for hypertension (Law *et al.* 1993). The offspring of families with essential hypertension have significantly higher AIx that the offspring of families with normal BP (Yasmin *et al.* 2004a).

Augmentation index and central PP are independent predictors of all-cause mortality in patients with end-stage renal disease (Safar *et al.* 2002; London *et al.* 2001), of cardiovascular events in patients undergoing percutaneus coronary intervention (Weber *et al.* 2005) and in hypertensive patients according to the CAFÈ study (Williams *et al.* 2006). At present, there is no study available where Tr (measured by systolic PWA) has been demonstrated to predict cardiovascular risk. However, the results of the direct measurement of the timing of the reflected waveform during angiography have been shown to predict cardiovascular and all-cause mortality in patients with chronic renal failure (Ueda *et al.* 2004a). It has been also reported that restenosis after percutaneus coronary stenting or after conventional balloon angiopgraphy is related to invasively measured inflection time (Ueda *et al.* 2004b).

Effect of physiologic and pharmacologic intervention on augmentation index

Changes in the physical properties of the arterial tree are also influenced by adverse lifestyle aspects such as smoking (Vlachopoulos *et al.* 2004a; Vlachopoulos *et al.* 2004b; Mahmud and Feely 2003), coffee consumption (Vlachopoulos *et al.* 2006a; Vlachopoulos *et al.* 2004b; Mahmud and Feely 2001), and excess alcohol consumption (Mahmud and Feely 2002a), which results in an abnormal increase in AIx.

Lifestyle modification, particular aerobic exercise and sodium restriction, are associated with improvement of arterial stiffness (Tanaka *et al.* 2005). Individuals with higher levels of habitual physical activity have reduced arterial stiffness (Boreham *et al.* 2004; Ferreira *et al.* 2002). Endurance exercise on a regular basis is associated with lower AIx in older men (Vaitkevicius *et al.* 1993). Moreover, AIx is inversely associated with cardiorespiratory fitness, measured by maximal oxygen consumption *per* kilogram (VO_2max/kg), in men without CVD (Binder *et al.* 2006)

A number of studies reported a beneficial effect of pharmacological treatment on AIx. Glyceryl trinitrate (Kelly *et al.* 1990), ACE-inhibitors (Ting *et al.* 1995), AT1 blockers (Mahmud and Feely 2002b) and sildenafil (Vlachopoulos *et al.* 2003) have been demonstrated to reduce AIx and central PP in different clinical settings. Moreover, in the CAFÈ study the investigators demonstrated that despite a similar reduction in brachial BP, amlodipine treatment for up to 4 years improves also central aortic BP in comparison with atenolol therapy (Williams *et al.* 2006). Recently, Dhakam *et al.* (2006) found that 6-week treatment with atenolol or eprosartan had a similar effect on peripheral BP, while eprosartan had a significantly greater effect on central SBP and central PP than atenolol. Moreover, AIx increased significantly after atenolol treatment.

In a placebo-controlled randomized study, AIx was reduced 6 hours after oral intake of vitamin C (Wilkinson *et al.* 1999). Recently, the positive effect on AIx after acute administration of C-vitamin was confirmed also by Mullan *et al*. (2002) in type 2 diabetic patients.

The value of arterial stiffness (measured by PWV or estimated from AIx) in reduction of cardiovascular events under treatment is not yet adequately demonstrated. In patients with end-stage renal failure the decrease of PWV, independently of BP changes, was an independent predictor of mortality (Guerin *et al.* 2001). Whether reduction of AIx and central PP is associated with less future cardiovascular events remains to be demonstrated. In this respect, the first results from CAFÈ study (Williams *et al.* 2006) are encouraging.

2.3.2.4. Diastolic pulse wave analysis

Diastolic PWA is based on a modified Windkessel model. The arterial pulse is recorded at the radial artery, but arterial stiffness is derived differently, based on assessment of diastolic decay, using the parameters of a 4-element Windkessel model (Chon *et al.* 1995). This method is implemented by the HDI/Pulse WaveCR-2000 Research Cardiovascular Profiling System (Hypertension Diagnostic Inc., Eagan, USA) and is used to derive information on the elasticity of both the proximal (large) and the distal (small) arteries. An exponential decay curve represents large (capacitative) artery elasticity, referred to as C1. The other component, referred to as C2, oscillatory elasticity or reflective elasticity, provides a measure of small artery elasticity (Oliver and Webb 2003). Studies have demonstrated strong correlation between C2 and AIx, which indicates that

C2 is, at least in part, a measure of arterial wave reflection (Rietzschel *et al.* 2001; Segers *et al.* 2001). Several authors have reported a link between endothelial function and C2 (Tao *et al.* 2007; Parvathaneni *et al.* 2002). C2 may provide a possible link with endothelium-mediated arterial tone (McVeigh *et al.* 2001), indicating relationship with functional changes in the vasculature.

There is no distinct anatomical separation between the small and the large arteries. The arterial pressure waveform is derived from the model of the systemic circulation representing a complex interaction of left ventricular stroke volume with the physical properties of the arterial three, and characteristics of the fluid present in the arterial system (Cohn *et al.* 1995).

Indices of diastolic pulse wave analysis and cardiovascular risk

This method has been applied to a number of at-risk populations. It has been suggested that large arteries are particularly sensitive to aging, while reflectance component is influenced by vascular diseases (Glasser *et al.* 1997). Studies have shown that C1 and C2 decrease with age and are gender-dependent (McVeigh *et al.* 1999). Smoking is associated with impaired C2 (McVeigh *et al.* 1997). Compared with healthy controls, C2 was reduced in patients with hypertension, CAD, peripheral arterial disease and diabetes (Cohn *et al.* 1995; Glasser *et al.* 1997; McVeigh *et al.* 1999; Kals *et al.* 2006b; Tao *et al.* 2007). In hypertensive patients, antihypertensive treatment is associated with a significant improvement of arterial elasticity (C1 and C2) (Shargorodsky *et al*. 2002; Resinik and Lester 2002). Moreover, preliminary results indicate that reduced C2 is associated with the future cardiovascular events in general population (Grey *et al.* 2003).

2.3.3. Arterial stiffness, inflammation and oxidative stress

The first known study showing the link between peripheral PP, as a surrogate measure of arterial stiffness, and CRP in general population was published by Abramson *et al.* (2002a). They demonstrated independent association between peripheral PP and CRP among apparently healthy subjects. An earlier study had reported association between essential hypertension and higher CRP levels but had not examined PP (Bautista *et al.* 2001). At the same time, another study demonstrated correlation of ICAM-1 and IL-6 with peripheral PP (Chae *et al.* 2001). Elevated CRP levels are associated with endothelial dysfunction, and normalisation of CRP levels over time is associated with a significant improvement in endothelial dependent forearm blood flow responses (Fichtlscherer *et al.* 2000). The association between AIx and endothelial function (Ravikumar *et al.* 2002) allows to suggest that inflammation may also affect arterial stiffness.

There is no study available demonstrating association between arterial stiffness, measure by PWA and oxidative stress. Increased oxidative stress may be an important mechanism in impaired endothelial function (Heitzer *et al.* 2001). Controversial data have been published on the relationship between oxLDL and peripheral SBP and DBP (Chrysohoou *et al.* 2007; Frostegård *et al.* 2003). Unfortunately, in both above studies the investigators did not calculate peripheral PP and its association with oxLDL. Elevated Hcy may stimulate oxidative stress and elastinolytic processes in arterial wall and modify elastin metabolism, by blocking the aldehyde groups in elastin, and thereby increase arterial stiffness (Celermajer *et al.* 1993; Demuth *et al.* 1998). The association between Hcy and AIx or PWV has been demonstrated in high risk subjects (Yasmin *et al.* 2004a; Bortolotto *et al.* 1999), whereas recent studies involving healthy middle-aged volunteers revealed no correlation between Hcy and PWV (de Bree *et al.* 2006; Woodside *et al.* 2004).

Further studies are needed to clarify the association between arterial stiffness, inflammation and oxidative stress.

2.4. Carotid artery intima-media thickness

High resolution B-mode ultrasound is a noninvasive method for examining the combined thickness of the carotid artery intima and media. IMT has been used in research settings to identify patients with subclinical atherosclerosis and it is considered a validated surrogate marker of systemic atherosclerotic disease (Salonen and Salonen 1993). The IMT of the carotid bulb and occurrence and size of plaque in the carotid artery are associated with the degree of carotid atherosclerosis measured at autopsy (Pignoli *et al.* 1987) and with atherosclerosis measured by coronary angiography (Hulthe *et al.* 1997). Longitudinal studies have shown relationship between IMT and future events, most frequently the incidence of cardiac events (myocardial infarction, angina pectoralis, coronary intervention) and cerebrovascular events (stroke or transient ischemic attack) (O'Leary *et al.* 1999; Chambless *et al.* 2000; Lorenz *et al.* 2007). Incidence of CAD, up to a mean IMT of 1 mm, women showed lower rates of adjusted annual events than men; above 1 mm, event rate for women was closer to that for men (Chambless *et al.* 1997). In another study (Salonen and Salonen 1993), a 0.1 mm increase in IMT was associated with the 11% increase in the risk of myocardial infraction. Moreover, antihypertensive treatment with calcium antagonist lacidipine slows down progression of IMT in hypertensive patients, independently of its BP action (Zanchetti *et al.* 2002).

Carotid IMT increases with age. Longitudinal estimates of the rate of progression of IMT have been demonstrated to be 0.005 mm (Sun *et al.* 2002) to 0.06 mm (Salonen and Salonen 1990) *per* year in asymptomatic people. Progression rate higher than 0.03 mm *per* year is associated with increased risk

of myocardial infraction and stroke (Belcaro *et al.* 2001; O'Leary *et al*. 1999). Gender is also an important determinant of IMT. Previous studies have been shown that men have higher IMT than women (Sun *et al.* 2002; Denarie *et al.* 2000). In addition, BP, total cholesterol, body mass index (BMI), smoking, i.e. traditional cardiovascular risk factors, are independently associated with IMT in middle-aged and older populations (Howard *et al.* 1993; Wilson *et al.* 1997). The results published in different studies about associations of novel cardio-vascular risk factors, e.g. CRP, oxLDL or Hcy, with IMT in asymptomatic persons are discordant. In an AIR study, plasma oxLDL was associated with IMT in clinically healthy persons (Hulthe and Fagerberg 2002). Controversial data have been published regarding the correlation between CRP and IMT in asymptomatic subjects (Elkind *et al.* 2005; Van der Meer *et al.* 2002). Recently, Durga *et al.* (2004) summarized the results of different studies on Hcy and IMT, and concluded that there exists no association between Hcy and IMT.

3. AIMS OF THE STUDY

The main goal of the present study was to investigate the impact of age, inflammation and oxidative stress related markers on arterial stiffness and carotid artery intima-media thickness.

The specific aims of the present study were the following:

1. To investigate the relationship between arterial stiffness, measured by the indices of systolic pulse wave analysis, and C-reactive protein in healthy subjects.

2. To determine the association of carotid artery intima-media thickness with oxidative stress-related markers in healthy subjects.

3. To compare plasma C-reactive protein and arterial stiffness, measured by the indices of systolic pulse wave analysis, in hypertensive subjects without any other traditional cardiovascular risk factors and in age- and sex-matched normotensive controls, as well as to assess the relationship between augmentation index and C-reactive protein.

4. To investigate the effect of extreme physical load on the inflammatory markers and to establish the impact of physical stress-induced acute inflammation on arterial elasticity, measured by the indices of diastolic pulse wave analysis.

5. To study the effect of age on augmentation index and on carotid artery intima-media thickness, and to test the hypothesis that while augmentation index is more important in younger subjects (≤50 years), carotid artery intima-media thickness is more useful in older subjects (>50 years).

4. SUBJECTS AND METHODS

4.1. Study subjects

4.1.1. Healthy subjects

The study population consisted of 175 healthy subjects in the age range 40 to 70 years. The subjects were studied at the Department of Cardiology, University of Tartu. The subjects were recruited between January 2002 and December 2004. All subjects passed a routine medical evaluation including a complete history and physical examination, electrocardiography, and blood tests. The exclusion criteria were the following: clinically overt CAD, or valve pathologies, arterial hypertension, cerebral and/or peripheral atherosclerotic disease, diabetes (fasting plasma glucose >6.4 mmol/l), malignancies, chronic degenerative diseases, endocrine pathologies, and according to a self-reported questionnaire: regular use of vitamins, vasoactive, antiinflammatory or steroid substances during the last two months. Subjects above the upper limit of the normal range of CRP (>5 mg/l) were also excluded from analysis as having apparently infection or inflammatory disorders. No dietary restrictions were imposed.

In paper I, 158 subjects aged 40 to 65 years and in paper III all healthy subjects from the study group were included.

4.1.2. Patients with uncomplicated essential hypertension and controls

4.1.2.1. Hypertensive patients

A total of 35 male and 7 female outpatients, aged 35–65 years, with untreated mild to moderate essential hypertension were included in the study (Paper II). All subjects who responded to the advertisement and in whom the inclusion criteria were met were recruited on a consecutive basis between September 2000 and May 2003 at the Department of Cardiology, University of Tartu. The diagnosis of hypertension was established on the basis of SBP ≥140 mmHg and /or DBP ≥90 mmHg measured during three different visits. Patients with previous antihypertensive treatment had been free of medication for at least 2 months. Twenty-nine patients had never been treated (69.0%), 6 (14.3%) had not been on antihypertensive treatment during the previous two months and 7 (16.7%) patients had not been treated during the last six months to three years.

All patients were non-smokers with a BMI of <30 kg/m^2. None of the patients displayed clinical evidence suggestive of CAD, based upon history, electrocardiography, exercise test and echocardiography. There was no left ventricular dysfunction on echocardiography (left ventricular ejection fraction <50%) or microalbuminuria on urine analysis. We excluded patients with diabetes (based upon glucose tolerance test), cerebrovascular disease, hypercholesterolemia (total cholesterol >6.5 mmol/l), renal diseases, other systemic diseases, recent/current infection (CRP >5mg/l) and anemia. Routine clinical, hematological and radiological examinations were performed to exclude secondary forms of hypertension. The subjects who were taking any medical preparations (including regular use of vitamin, vasoactive, antiinflammatory or steroid substances) were not included. No dietary restrictions were imposed.

4.1.2.2. Controls

Thirty-five male and 7 female healthy control subjects were recruited from general population and were matched with the patients for age and sex. All controls demonstrated normal findings at physical and biochemical examinations, and had normal BP values (SBP lower than 138 mmHg and DBP lower than 88 mmHg). All controls were non-smokers with a BMI of <30 kg/m^2. None of them displayed clinical evidence suggestive of CAD or heart failure based upon history, electrocardiography, exercise test and echocardiography. The subjects with hypercholesterolemia (total cholesterol >6.5 mmol/l) and above the upper limit of the normal range of CRP (>5 mg/l) were also excluded from analysis. The subjects who were taking any medical preparations (including regular use of vitamin, vasoactive, antiinflammatory or steroid substances) were not included. No dietary restrictions were imposed.

4.1.3. Army cadets

Seven well-trained male army cadets, aged 21–29 years, were examined during an international military combat course (ERNA, Estonia) (Paper IV). All controls demonstrated normal findings at physical and biochemical examinations, and had normal BP values (SBP lower than 140 mmHg and DBP lower than 90 mmHg). None of the participants showed any signs or symptoms of CVD (based upon history, electrocardiography and exercise test), infection or inflammatory disorders. The subjects above the upper limit of the normal range of CRP (>5 mg/l) were also excluded from analysis as having apparently infection or inflammatory disorders. The subjects reported that they had not used any vitamin, vasoactive or anti-inflammatory medication during the previous two months. No dietary restrictions were imposed

4.2. Methods

4.2.1. Study Protocol

Healthy subjects were studied and plasma samples were collected between 8:00 and 10:00 am, after an overnight fast and abstinence from tobacco, alcohol, tea or coffee. After 15 minutes of rest, BP was measured, electrocardiography and systolic PWA were performed. Thereafter, blood samples were drawn from the antecubital vein for measurement of plasma CRP, glucose, total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, oxLDL, Hcy, white blood cell count (WBC), red blood cell count (RBC), hematocrit, haemoglobin and platelets. IMT examination was made at the same visit as blood was sampled, or with an interval of a few weeks. Height and weight were recorded, and BMI was calculated (Papers I and III).

Hypertensive patients and sex-age matched control subjects were seen 4 weeks before they entered the study. During this time repeated measurements of BP, electrocardiography, exercise stress test, glycose tolerance test, echocardiography and ultrasound investigation of the renal arteries were performed. When the subjects met the study criteria, they were enrolled in the study. On the study morning plasma samples were collected between 8:00 and 10:00 am, after an overnight fast and abstinence from alcohol, tea or coffee. After 15 minutes of rest, BP was measured and systolic PWA was performed. Thereafter, blood samples were drawn from the antecubital vein for measurement of plasma CRP, fibrinogen, glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, WBC, RBC, hematocrit, haemoglobin and platelets. Height and weight were recorded, and BMI was calculated (Paper II).

Male army cadets were studied and plasma samples were collected 48 hours before the competition between 8:00 and 10:00 am, after an overnight fast and abstinence from tobacco, alcohol, tea or coffee. After 15 minutes of rest, BP was measured, and electrocardiography and diastolic PWA were performed Thereafter, blood samples were drawn from the antecubital vein for measurement of plasma CRP, glucose, creatine kinase, WBC, RBC, haematocrit, haemoglobin and platelets. VO_2max/kg was measured by incremental running exercise test on the same day 2–4 h after breakfast. A military combat course of 3.5-days (total 84.5 hours) involved walking, jogging and special military combat activities (approximate total distance 135 km). During the race the cadets had to avoid the reconnaissance patrol who were assigned the task of making the race as difficult as possible. The sleeping time during the race was limited to 240 minutes *per* night. A participant carried a backpack with special equipment and an automatic rifle with a combined weight of 25 kg. During the race food and drinks were provided as part of the equipment and additional drinks were available at each checkpoint. The covered distance and the precise

location of each subject were monitored using a global positioning system. Heart rate was monitored continuously and stored at 60sec intervals using a telemetry system (Sporttester Polar S810, Finland). Twenty-four hours after the competition (24-hour recovery period) all blood tests and diastolic PWA were repeated under the conditions described previously.

4.2.2. Blood pressure measurement

SBP and DBP were measured in both arms at least twice with a mercury sphygmomanometer (Riester, Germany), with the subject seated for 10 minutes. The first and the fifth Korotkoff sounds were recorded to determine peripheral SBP and peripheral DBP. Peripheral PP was calculated as the difference between peripheral SBP and peripheral DBP. The mean of the two readings was used in analysis (Paper I–IV).

4.2.3. Systolic pulse wave analysis

Systolic PWA was assessed by a sphygmocor apparatus (SphygmoCor Px, Version 7.0, AtCor Medical, Australia). After at least 15 min of rest in a quiet temperature-controlled room, the waveforms of peripheral pressure were recorded over 10 seconds from the radial artery of the dominant arm at the wrist employing a high fidelity micromanometer (SPT-301B, Millar Instruments, USA). Using a validated transfer function (Karamanoglu *et al*. 1993; Chen *et al.* 1997), the corresponding ascending aortic waveforms were then generated, from which central BP, AIx and Tr were calculated. Because heart rate is a major confounder of AIx (Wilkinson *et al*. 2000), the software also generates an AIx, corrected to a heart rate of 75 beats *per* minute (AIx HR 75). PWA recordings were made in duplicate and the mean of the results was used in analysis (Paper I–III).

4.2.4. Diastolic pulse wave analysis

Diastolic PWA was performed after 15 min of rest in a supine position in a quiet temperature-controlled room. Peripheral BP and the arterial waveform were measured in the dominant arm by the Cardiovascular Profiling Instrument (HDI/Pulse Wave CR-2000, Hypertension Diagnostics Inc., USA). The tonometer was applied to the patient's radial artery at the wrist overlying the radial bony prominence. The cuff for BP measurement was placed on the contralateral arm and inflated concurrently with pulse waveform recording for calibration. The elasticity indices of the arteries (C1 and C2) were quantified

during the diastolic portion of the cardiac cycle (mean of 30 second recording). Heart rate, MAP and stroke volume were also calculated from the radial pressure waveform using the HDI/Pulse Wave CR-2000 software. Haemodynamic and PWA recordings were made in duplicate and the mean of the results was used in analysis (Paper IV).

4.2.5. Incremental running exercise test

Incremental running exercise test on the treadmill was performed according to a standard protocol test using the ParvoMedics Truemax 2400 Metabolic Measurement System (ParvoMedics, USA). The subjects were required to meet two of the three standard criteria for having achieved VO_2max (heart rate ≥age-predicted maximum heart rate, respiratory exchange ratio ≥1.10, rating of perceived exertion ≥19). Prior to testing, the gas analyser was calibrated with standard gases of known concentration (Paper IV).

4.2.6. Ultrasound examination of carotid artery intima-media thickness

Measurements of IMT were performed with an ultrasound scanner (LOGIQ 9, GE Medical Systems, UK), using a 12 MHz transducer. One physician (Dr. Kai Ulst) carried out all ultrasound examinations. Scanning was videotaped on a super-VHS for further analysis. IMT was determined as described previously (Liu *et al.* 2004). Longitudinal images from 3 projections (anterolateral, lateral, and posterolateral) were measured for the common carotid artery, the carotid bulb, and the internal carotid artery. Measurements were performed at a total of 28 sites, both the far wall and the near wall of the arterial segments, 1 cm right and left distal of the common carotid artery, carotid bulb, and 1 cm proximal of the internal carotid artery. All three projections of the common carotid artery and the carotid bulb, and a single angle of the internal carotid artery with the best visibility were used. Mean, maximum, and minimum IMT were derived from each measurement. The average of all mean IMT measurements over 28 (or fewer) sites was chosen as the outcome variable.

IMT examination was made at the same visit as blood was sampled, or with an interval of a few weeks. For technical and anatomic reasons, the data of IMT measurements for 23 patients were missing (Paper III).

4.2.7. Laboratory analyses

WBC, RBC, hematocrit, haemoglobin, platelets, plasma glucose, total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels were determined immediately by standard laboratory methods, using certified assays, in a local clinical laboratory (United Laboratories of Tartu University Hospital). Lipid levels were measured by the Hitachi 912 analyser (Roche Diagnostics, Germany). WBC and RBC, haematocrit, haemoglobin and platelets were measured using the Sysmex XE 2100 autoanalyser (Sysmex Corporation, Japan) (Papers I–IV).

CRP was determined immediately by a validated high-sensitivity assay using a latex particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Germany) with the automated analyser Hitachi 912 (Papers I–IV). Fibrinogen was measured by the clotting method after Clauss using the Stago Compact analyser (Diagnostica Stago, France) (Paper II). OxLDL levels were measured using an enzyme-linked immunosorbent assay kit (two monoclonal antibodies directed against separate antigenic determinants on the oxidized apolipoprotein B molecule) (Mercodia AB, Sweden) using a photometer Sunrise (Tecan Austria GmbH, Austria) (Paper III). Hcy was measured with the enzyme immunoassay method (Axis-Shield Diagnostics Ltd., UK) using a photometer Sunrise (Tecan Austria GmbH, Austria) (Paper III).

4.2.8. Statistical analysis

Statistical analyses were conducted using the software R, version 1.9.0 for Windows (Papers I–II), statistical package Statistica 7.0 (StatSoft, USA) (Paper III) or the SPSS version 11.0 for windows (SPSS, USA) (Paper IV). All data were tested for normality using the Kolmogorov-Smirnov test. Variables with a non-normal distribution were log-transformed for further analysis. Continuous variables are presented as mean ±SD for normally distributed data and as medians with an inter-quartile range for non-normally distributed data and as frequencies (n) or percentages (%) for categorical variables.

In Papers I–IV, the data were analysed using the unpaired two-tailed *t*-test, Fisher's exact test, Pearson correlation and multiple regression analysis. The predictors for subsequent multiple regression analysis were selected on the basis of simple correlation analysis, and from among the variables known or likely to be associated with the dependent variable.

In Paper II, the baseline characteristics of the cases and of the controls were analysed using the unpaired *t*-test for the normally distributed data and the Mann-Whitney *U*-test for the non-normally distributed data.

In Paper III, a change in r^2 indicates the change for each parameter in percentages. The differences in the oxLDL tertiles or CRP groups were tested by two-way ANOVA and by the unpaired *t*-test.

In Paper IV, the data were analysed using the paired-samples *t*-test and the Wilcoxon paired test.

Significance was defined as $p < 0.05$.

5. RESULTS

5.1. C-reactive protein and its association with the indices of systolic pulse wave analysis in healthy persons (Paper I)

5.1.1. Baseline characteristic of the study subjects

The baseline clinical characteristics of the 158 study subjects are summarized in Table 1.

Table 1. Baseline characteristics of the study subjects

Variable	Female N=83	Male N=75	p
Age, y	48.9±5.6	50.6±6.6	0.07
BMI, kg/m^2	25.1±4.2	25.6±3.3	0.4
Height, m	1.7±0.06	1.8±0.06	<0.001
Weight, kg	68.2±11.7	80.6±11.0	<0.001
Peripheral SBP, mmHg	116.7±12.4	120.2±11.3	0.03
Peripheral DBP, mmHg	76.6±6.9	78.2±7.7	0.2
Peripheral PP, mmHg	39.6±8.7	41.9±8.1	0.07
MAP, mmHg	91.3±8.5	92.4±8.5	0.4
Central SBP, mmHg	107.5±11.6	109.1±10.6	0.4
Central DBP, mmHg	77.7±7.0	79.1±7.9	0.2
Central PP, mmHg	29.8±7.3	30.1±7.4	0.8
AIx, %	24.9±9.8	16.8±12.5	<0.001
AIx HR 75, %	25.1±8.5	12.9±10.8	<0.001
Heart rate, beats/min	75.2±10.7	67.2±10.2	<0.001
Tr, ms	140.0±11.1	148.5±10.4	<0.001
Total cholesterol, mmol/l	5.9±1.0	5.7±1.2	0.2
HDL cholesterol, mmol/l	1.6±0.4	1.4±0.4	<0.001
LDL cholesterol, mmol/l	3.8±0.9	3.7±1.0	0.8
Triglycerides, mmol/l	1.1±0.6	1.4±1.5	0.1
Current smoking, n (%)	14(16.7)	13(16.7)	1.0
Cigarette, n/day	10.4±3.8	15.3±6.5	0.02
Smoking history, y	26.9±6.1	29.0±5.3	0.35
CRP, mg/l	0.92 (0.5;1.58)	0.87 (0.62;1.69)	0.4
WBC, x10^{9}l	5.0±1.2	5.37±1.2	0.09
RBC, x 10^{12}l	4.4±0.3	4.7±0.3	<0.001
Haemoglobin, g/l	129.8±9.6	144.8±9.6	<0.001
Hematocrit, %	38.5±2.5	42.3±2.5	<0.001
Platelets, x10^{9}l	231.6±53.7	223.4±45.0	0.3
Glucose, mmol/l	4.9±0.5	5.2±0.6	<0.001

5.1.2. Distribution of C-reactive protein according to cardiovascular risk

To detect cardiovascular risk according to CRP, the subjects were stratified into two groups (Table 2).

Table 2. Baseline characteristics according to CRP distribution

Variable	CRP <1 mg/l N=86 (41 male, 45 female)	CRP ≥1 mg/l N=72 (34 male, 38 female)	p	r
Age, y	48.2±5.5	50.9±5.9	0.003	0.29†
Weight, kg	70.9±10.8	77.6±14.3	0.002	0.37†
Height, m	1.7±0.1	1.7±0.1	0.2	–0.01
BMI, kg/m^2	24.1±3.0	26.8±4.1	<0.001	0.45†
Peripheral SBP, mmHg	116.6±10.1	119.0±12.0	0.2	0.20*
Peripheral DBP, mmHg	76.5±6.6	78.1±7.4	0.2	0.20*
Peripheral PP, mmHg	40.2±7.2	41.0±8.8	0.5	0.11
MAP, mmHg	90.4±7.3	93.0±8.3	0.039	0.26†
Central SBP, mmHg	106.1±9.3	110.1±11.1	0.02	0.29†
Central DBP, mmHg	77.4±6.7	79.0±7.5	0.2	0.20*
Central PP, mmHg	28.7±6.0	31.1±7.9	0.035	0.23†
AIx, %	18.1±12.6	24.5±9.9	<0.001	0.28†
AIx HR 75, %	16.2±12.1	22.9±9.6	<0.001	0.29†
Heart rate, beats/min	70.9±9.8	71.7±12.4	0.7	0.00
Tr, ms	145.1±12.1	142.8±10.9	0.2	–0.15
Total cholesterol, mmol/l	5.7±1.0	5.9±1.0	0.2	–0.02
HDL cholesterol, mmol/l	1.6±0.4	1.4±0.3	0.008	–0.26†
LDL cholesterol, mmol/l	3.6±1.0	3.9±0.9	0.06	0.03
Triglycerides, mmol/l	1.1±0.6	1.0±0.6	0.04	0.21*
Current smoking, %	17.4	15.3	0.8	
WBC, x10^{9}l	5.0±1.1	5.4±1.4	0.04	0.25†
RBC, x 10^{12}l	4.5±0.4	4.6±0.3	0.5	0.15
Haemoglobin, g/l	136.5±13.0	137.3±11.3	0.7	0.08
Hematocrit, %	40.2±3.3	40.4±2.9	0.7	0.09
Platelets, x10^{9}l	224.3±49.5	232.4±48.2	0.3	0.06
Glucose, mmol/l	5.0±0.5	5.1±0.5	0.2	0.12

r – Pearson correlation coefficient between log(CRP) and variable (*p<0.05, †p<0.01).

The AIx was significantly higher in patients with CRP levels above 1 mg/l (24.5±9.9% vs 18.1±12.6%, p<0.001, r=0.28). When the two CRP groups were compared, significant differences were revealed in central SBP, central PP, and MAP. No differences were found between the groups regarding peripheral SBP, peripheral PP or Tr.

5.1.3. Multivariate relations between the indices of systolic pulse wave analysis and other variables

In correlation analysis, significant association were found between AIx and plasma log(CRP) (Figure 2).

In multiple regression analysis (Table 3; r^2=0.64, p<0.0001), AIx as the dependent variable correlated significantly with female gender, age, height, heart rate, MAP, WBC (p=0.01) and log(CRP) (p=0.026). Adjusting additionally for weight, smoking, plasma glucose, LDL cholesterol, HDL cholesterol and triglycerides did not have any significant effect on the fitted regression model.

Table 3. Results of multiple regression analysis with AIx as the dependent variable

Parameter	Regression coefficient	Standard error	p
Age, y	0.56	0.11	<0.001
Gender, female	9.57	1.82	<0.001
Height, m	–42.23	9.68	<0.001
Heart rate, beats/min	–0.58	0.06	<0.001
WBC, x10^9l	1.29	0.52	0.01
Log(CRP)	1.93	0.86	0.026
MAP, mmHg	0.42	0.08	<0.001
Smoking	2.78	1.67	0.1

r^2=0.64, p<0.0001 for the entire study group (n=158 subjects).

Table 4. Results of multiple regression analysis with central PP as the dependent variable

Parameter	Regression coefficient	Standard error	p
Age, y	0.30	0.09	<0.001
Gender, female	1.65	1.56	0.3
Height, m	–5.64	8.08	0.5
Heart rate, beats/min	–0.22	0.05	<0.001
WBC, x10^9l	–0.07	0.44	0.9
Log(CRP)	1.38	0.50	0.006
Glucose, mmol/l	2.30	0.96	0.02
Smoking	0.94	1.40	0.5

r^2=0.28, p<0.0001 for the entire study group (n=158 subjects).

In multiple regression analysis, central PP correlated significantly with age, heart rate, log(CRP), and serum glucose (Table 4; r^2=0.28, p<0.0001). The same model was used also for peripheral PP, multiple regression analysis revealed no correlation between log(CRP) and peripheral PP (p=0.09) as the dependent variable.

Aortic Tr as the dependent variable correlated significantly only with height and MAP, no correlation revealed for age, gender, heart rate, WBC ($p=0.7$), log(CRP) ($p=0.4$) and smoking in multiple regression analysis ($r^2=0.28$, $p<0.0001$).

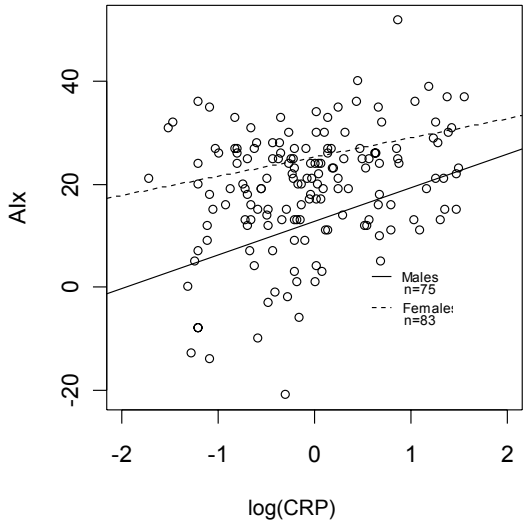

Figure 2. Scatter plot of log(CRP) and AIx together with separate regression lines for males and females: $r=0.37$, $p<0.01$ for males and $r=0.28$, $p=0.01$ for females.

5.2. C-reactive protein and augmentation index in hypertensive subjects and in normotensive controls (Paper II)

5.2.1. Baseline characteristics of the study subjects

Comparison of the hypertensive and of the control group (separately for males and females) revealed no significant difference in age, height, heart rate, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, RBC, hematocrit, haemoglobin, platelets or fibrinogen. Among the male patients, plasma concentrations of glucose, CRP and WBC were significantly higher for the hypertensive than for the healthy controls. Among the females, only CRP was significantly higher for the hypertensive than for the healthy controls (Table 5).

Table 5. Clinical characteristics of the study group

Variable		Patients N=35 M/7 F	Controls N=35 M/7F	p
Age, y	M	53.7±7.2	51.8±7.9	0.3
	F	49.6±5.7	47.6±5.9	0.5
BMI, kg/m^2	M	27.1±1.9	25.7±2.6	0.008
	F	25.1±2.8	24.5±3.2	0.7
Height, m	M	1.79±0.06	1.77±0.05	0.2
	F	1.63±0.07	1.69±0.08	0.2
SBP, mmHg	M	148.3±10.9	116.7±12.1	<0.001
	F	153.7±8.7	118.9±5.3	<0.001
DBP, mmHg	M	96.6±6.9	74.7±7.6	<0.001
	F	95.1±6.3	74.9±6.2	<0.001
MAP, mmHg	M	114.2±7.9	88.1±8.7	<0.001
	F	116.9±2.8	89.9±5.7	<0.001
Heart rate, beats/min	M	67.5±8.9	66.0±10.0	0.5
	F	77.0±10.3	77.7±8.6	0.9
AIx, %	M	20.2±12.3	11.4±13.7	0.006
	F	28.9±8.2	16.7±9.8	0.02
AIx HR 75, %	M	16.7±10.6	7.1±11.8	<0.001
	F	29.7±5.1v	17.9±6.8	0.003
Tr, ms	M	142.9±11.3	152.0±11.3	0.001
	F	129.4±6.6	143.9±9.3	0.005
CRP, mg/l	M	1.27 (0.94;2.17)	0.74 (0.52;1.02)	<0.001
	F	1.22 (0.86;1.52)	0.69 (0.58;0.82)	0.02
WBC, $x10^9 l$	M	5.6±1.4	5.0±0.8	0.02
	F	5.0±1.5	5.0±0.6	0.9
Fibrinogen, g/l	M	2.9±0.6	2.8±0.4	0.6
	F	2.9±0.4	3.0±0.4	0.7
Glucose, mmol/l	M	5.5±0.4	5.2±0.4	0.03
	F	5.1±0.5	4.9±0.5	0.4
Total cholesterol, mmol/l	M	5.5±0.7	5.2±0.7	0.07
	F	5.0±0.6	5.3±0.8	0.4
LDL cholesterol, mmol/l	M	3.6±0.7	3.5±0.7	0.3
	F	2.9±0.6	3.2±0.6	0.3
Triglycerides, mmol/l	M	1.4±0.7	1.2±0.5	0.08
	F	0.9±0.4	1.1±0.6	0.5
HDL cholesterol, mmol/l	M	1.3±0.3	1.4±0.3	0.6
	F	1.6±0.5	1.6±0.3	0.9

M, male subjects; F, female subjects.

5.2.2. Inflammatory markers and the indices of systolic pulse wave analysis

For the whole study group, plasma log(CRP) correlated significantly with peripheral SBP and central SBP ($r=0.50$, $p<0.001$ and $r=0.52$, $p<0.001$), peripheral DBP and central DBP ($r=0.42$, $p<0.001$ and $r=0.43$, $p<0.001$), and with peripheral PP and central PP ($r=0.38$, $p<0.001$ and $r=0.42$, $p<0.001$) in univariate linear correlation analysis. Also, significant correlations were found with age ($r=0.25$, $p=0.02$), BMI ($r=0.38$, $p<0.001$), AIx ($r=0.33$, $p=0.002$), AIx HR 75 ($r=0.40$, $p<0.001$) and Tr ($r=–0.34$, $p=0.002$).

For the whole study group, plasma fibrinogen correlated significantly with age ($r=0.36$, $p=0.001$), peripheral PP and central PP ($r=0.23$, $p=0.03$ and $r=0.27$, $p=0.01$), AIx ($r=0.23$, $p=0.04$), AIx HR 75 ($r=0.29$, $p=0.01$), and weakly with Tr ($r=–0.21$, $p=0.05$).

WBC correlated significantly, for the whole study group, with peripheral SBP and central SBP ($r=0.26$, $p=0.02$ and $r=0.30$, $p=0.006$), peripheral PP and central PP ($r=0.25$, $p=0.02$ and $r=0.33$, $p=0.002$) and AIx HR 75 ($r=0.30$, $p=0.006$). There was also weak but nonsignificant correlation with Tr ($r=–0.20$, $p=0.07$).

5.2.3. Multivariate relations between the indices of systolic pulse wave analysis and other variables

Mean AIx and Tr were significantly different in the hypertensive group in comparison with the control group (Table 5). The data of all study subjects were used in multivariate regression analysis for AIx as the dependent variable. MAP, age, gender, height, heart rate and log(CRP) were included in the model as the known determinants of AIx. As the model was adjusted for MAP, that can be used to distinguish hypertensive patients from the controls, no additional adjusting was needed for patients and controls. In the multiple regression model, AIx correlated positively with age, female gender, MAP and log(CRP), and negatively with heart rate and height (Table 6; $r^2=0.75$, $p<0.001$). After further adjustment for plasma glucose, neither LDL cholesterol and weight nor fibrinogen affected the fitted regression model. In multivariate analysis for the hypertensive group only, with AIx as the dependent variable, log(CRP) ($p=0.04$) was shown similarly to be the predictor of AIx when adjusting for the other variables as sex, age, heart rate, height and MAP ($r^2=0.77$, $p<0.001$).

Table 6. Results of multiple regression analysis with AIx as the dependent variable in whole study group

Parameter	Regression coefficient	Standard error	p
Age, y	0.73	0.12	<0.001
Gender, female	13.25	3.01	<0.001
Height, m	–31.98	13.67	0.02
Heart rate, beats /min	–0.69	0.09	<0.001
MAP, mmHg	0.31	0.06	<0.001
Log(CRP)	6.99	3.24	0.03

r^2=0.75, p<0.001 for the entire study group (n=84 subjects).

As there was strong correlation between log(CRP) and WBC (r=0.30, p=0.006), we performed another multivariate regression analysis for AIx as the dependent variable. In this model, AIx was positively correlated with age, female gender, MAP and WBC, and negatively with heart rate and height (Table 7; r^2=0.76, p<0.001).

Tr also showed negative correlation with log(CRP) (r=–0.34, p=0.002) for the whole study group. After adjusting Tr for MAP, heart rate, height, age and sex in the multiple regression model, which are all known determinants for Tr, no correlation was revealed between log(CRP) and Tr (p=0.35) as the dependent variable (r^2=0.48, p<0.001). There was no association found between Tr and log(CRP) when correlation performed separately for the controls and for the hypertensive patients (Figure 3).

Table 7. Results of multiple regression analysis with AIx as the dependent variable in whole study group

Parameter	Regression coefficient	Standard error	p
Age, y	0.75	0.11	<0.001
Gender, female	13.52	2.97	<0.001
Height, m	–30.33	13.46	0.03
Heart rate, beats/min	–0.68	0.09	<0.001
MAP, mmHg	0.34	0.05	<0.001
WBC, $x10^9$1	1.76	0.67	0.01

r^2=0.76, p<0.001 for the entire study group (n=84 subjects).

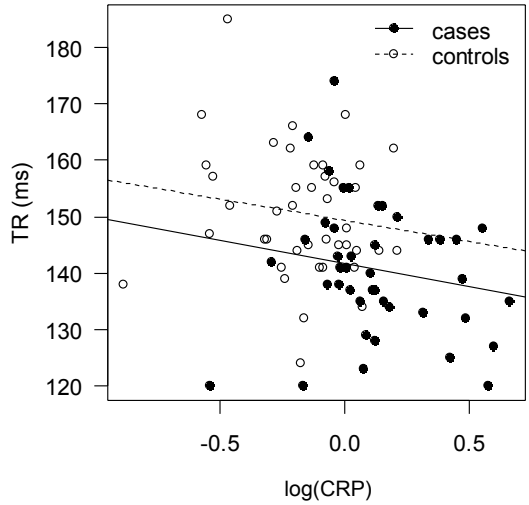

Figure 3. Scatter plot of log(CRP) and Tr together with separate regression lines for the controls (r=–0.15, p=0.34) and for hypertensive subjects (r=–0.18, p=0.26).

5.3. Association between age, inflammatory and oxidative stress- related markers with augmentation index and carotid artery intima-media thickness (Paper III)

5.3.1. Baseline characteristics of the study subjects

The characteristics of the 175 healthy subjects included in the study are summarized in Table 8.

5.3.2. Associations between the indices of inflammation and oxidative stress

The results of univariate correlation analysis for oxLDL, log(CRP) and log(Hcy) are shown in Table 9. The oxLDL was significantly correlated with peripheral and central SBP and DBP, MAP, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, log(triglycerides) and log(IMT).

Compared with the oxLDL tertiles, there occurred a significant increase in IMT (p=0.003). However, although AIx HR 75 was significantly increased in comparison with the first and the second oxLDL tertiles, there occurred no further significant increase in AIx HR 75 in the third oxLDL tertile (Figure 4A).

Table 8. Baseline characteristics of the study subjects

Variable	Study subjects (n= 175)
Age, y	49.7±6.2
Gender, male/female	88/87
BMI, kg/m^2	25.3±3.7
Smokers, n	27
Peripheral SBP, mmHg	118.1±10.8
Peripheral DBP, mmHg	76.8±7.0
Peripheral PP, mmHg	41.3±8.1
MAP, mmHg	91.4±7.8
Central SBP, mmHg	108.2±10.4v
Central DBP, mmHg	77.7±7.1
Central PP, mmHg	30.5±7.5v
Heart rate, beats/min	70.8±11.3
AIx, %	21.2±11.9
AIx HR 75, %	19.2±11.4
Tr, ms	143.9±11.3
Tr adjusted for height, ms	11.9±0.9
IMT, mm	0.74 (0.68;0.82)
Total cholesterol, mmol/l	5.6±1.0
LDL cholesterol, mmol/l	3.6±0.8
Triglycerides, mmol/l	1.0 (0.73;1.47)
HDL cholesterol, mmol/l	1.5±0.4
Glucose, mmol/l	5.0 (4.7;5.4)
CRP, mg/l	0.96 (0.58;1.74)
OxLDL, U/l	133.1±46.7
Hcy, µmol/l	8.4 (7.1;10.1)

Log(CRP) correlated significantly with age, weight, BMI, WBC, log(triglycerides), HDL cholesterol, AIx, AIx HR 75, Tr and central SBP. The log(CRP) correlated only weakly with central PP (r=0.14, p=0.065) and with log(IMT) (r=0.15, p=0.06). After adjusting IMT for age, the correlation between log(CRP) and log(IMT) disappeared (r=0.05, p=0.5). In this study the subjects were divided into three groups according to the cut-off values of CRP predicting cardiovascular risk as being low, moderate or high. For IMT, a significant increase was detected only between the first and the second CRP groups (Figure 4B). For AIx HR 75 a significant increase was demonstrated between the first and the second CRP groups (p=0.04), and between the first and the third CRP groups (p=0.002).

Table 9. Univariate correlation analysis of log(CRP), log(Hcy), and oxLDL

Variable	Log(CRP)	Log(Hcy)	oxLDL
Age	0.26†	0.30†	0.01
Gender	–0.05	–0.36†	0.07
Weight	0.31†	0.10	0.13
BMI	0.39†	–0.08	0.20†
WBC	0.18*	–0.0006	0.017
Log(glucose)	0.06	0.13	0.09
Total cholesterol	0.008	–0.14	0.60†
Log(triglycerides)	0.19*	0.10	0.38†
HDL cholesterol	–0.24†	0.01	–0.19*
LDL cholesterol	–0.10	–0.10	0.70†
Log(CRP)	.	0.13	0.03
Log(Hcy)	0.13	.	–0.14
OxLDL	0.03	–0.14	.
Peripheral SBP	0.11	0.16*	0.17*
Peripheral DBP	0.10	–0.01	0.23†
MAP	0.14	0.05	0.23†
Peripheral PP	0.06	0.23†	0.02
AIx	0.21†	0.02	0.07
AIx HR 75	0.24†	–0.09	0.13
Tr	–0.15*	0.11	–0.12
Tr adjusted for height	0.13	0.10	0.07
Central SBP	0.17*	0.15*	0.17*
Central DBP	0.11	–0.02	0.24†
Central PP	0.14	0.23†	0.01
Log(IMT)	0.16	0.17*	0.24†

*$p<0.05$, †$p<0.01$.

The log(Hcy) correlated significantly with age, gender, peripheral and central SBP, peripheral and central PP and log(IMT). After adjusting IMT for age, the correlation between log(Hcy) and log(IMT) disappeared ($p=0.6$).

The log(IMT) was significantly correlated with gender, age, height, weight, WBC, log(glucose), log(triglycerides), HDL cholesterol, LDL cholesterol, log(Hcy), oxLDL, MAP, Tr adjusted for height, and AIx. All these variables were entered in a stepwise linear regression model. Log(IMT) as the dependent variable correlated positively only with age, weight, WBC, oxLDL and Tr adjusted for height, and negatively with heart rate (Table 10; $r^2= 0.41$, $p<0.001$).

The AIx was significantly correlated with gender, age, height, weight, log(CRP), MAP, heart rate, Tr and log(IMT). In the stepwise linear regression model, AIx as the dependent variable correlated positively only with age, gender, MAP, weight and log(CRP) and negatively with heart rate and height (Table 11; $r^2=0.63$, $p<0.001$).

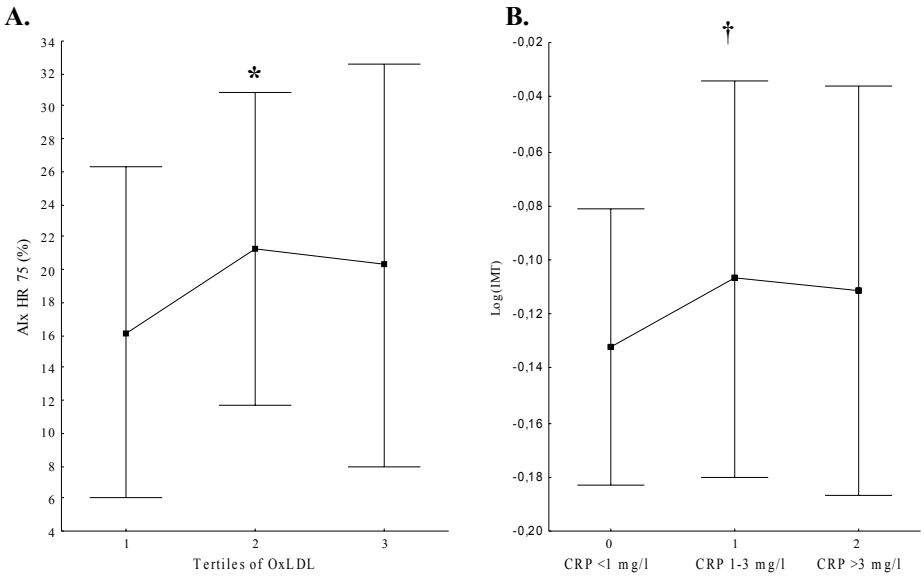

Figure 4. A. Mean (±SD) AIx HR 75 in different oxLDL tertiles. **B.** Mean (±SD) log(IMT) in different CRP groups.

* p=0.008 AIx HR 75 differences between the first and the second oxLDL tertiles.
† p=0.03 Log(IMT) differences between the first and the second CRP groups.

Table 10. Stepwise regression analysis for IMT

Parameter	Regression coefficient	Standard error	p	r^2 change (%)
Age, y	0.004	0.0007	<0.0001	19
Weight, kg	0.0008	0.0004	0.03	6
WBC, $x10^9$ l	0.01	0.003	0.001	5
OxLDL, U/l	0.0003	0.0001	0.006	3
Heart rate, beats/min	–0.001	0.0004	0.008	3
Height adjusted Tr, ms	0.02	0.005	0.0007	5

r^2= 0.41, p<0.001 (stepwise regression analysis of 152 subjects).

Table 11. Stepwise regression analysis for AIx

Parameter	Regression coefficient	Standard error	p	r^2 change (%)
Age, y	0.45	0.10	<0.0001	6
Gender, female	7.99	1.73	<0.0001	5
Height, m	–38.6	9.96	0.0002	18
MAP, mmHg	0.53	0.08	<0.0001	12
Heart rate, beats/min	–0.58	0.06	<0.0001	20
Weight, kg	–0.14	0.06	0.02	1
Log(CRP)	6.02	1.77	0.0008	2

r^2= 0.63, p<0.001 (stepwise regression analysis of 175 subjects).

5.3.3. Age and its associations with augmentation index and carotid artery intima-media thickness

There was strong correlation between age and AIx (r=0.3, p<0.001), and between age and log(IMT) (r=0.44, p<0.001). After dividing the patients into two groups, those ≤50 years and those >50 years of age, there occurred a significant correlation between AIx and age ≤50 years (r=0.33, p=0.001) and between log(IMT) and age >50 years (r=0.40, p=0.001). The significant correlation disappeared between AIx and age >50 years (r=0.17, p=0.13) and between log(IMT) and age ≤50 years (r=0.08, p=0.44) (Figure 5).

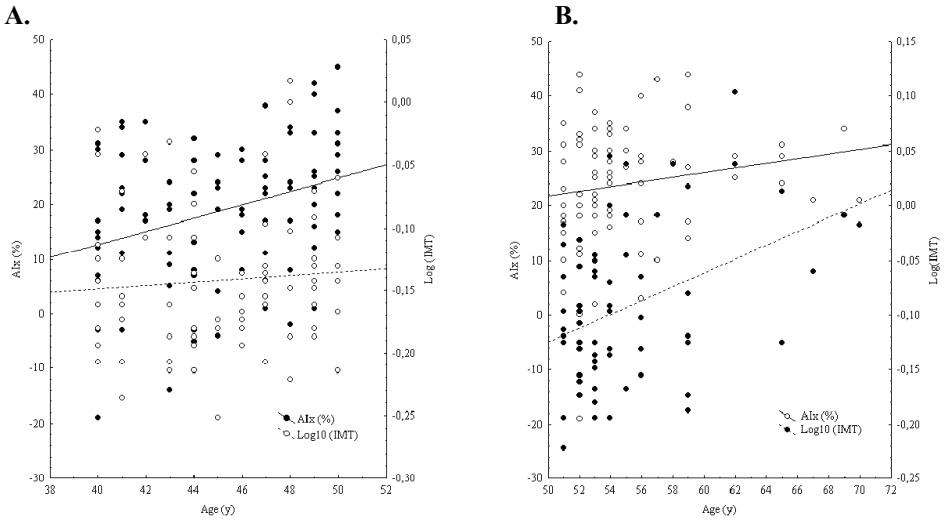

Figure 5. A. Black circles and the solid line indicate the correlation between age and AIx (n=95; r=0.33, p=0.001), and white circles and the dashed line indicate the correlation between age and log(IMT) (n=85 r=0.08, p=0.4) in ≤50 year-old subjects.
Figure 5. B. White circles and the solid line indicate the correlation between age and AIx (n=80; r=0.17, p=0.13), and black circles and the dashed line indicate the correlation between age and log(IMT) (n=67; r=0.40, p=0.001) in >50 year-old subjects.

5.4. Association between arterial elasticity and C-reactive protein at extreme physical load (Paper IV)

5.4.1. Baseline characteristics of the study subjects

The individual and mean values of the biochemical markers for the cadets before and after the race are presented in Tables 12 and 13. There was a 58% increase in plasma creatine kinase (p=0.002) and a 57% increase in plasma CRP (p=0.03) after the competition in comparison with the baseline values. The post-race values of plasma glucose, haemotocrit, haemoglobin, RBC and platelets did not differ significantly from the baseline values. Circulating levels of WBC remained unchanged after the race. SBP, DBP and MAP did not change significantly when compared with the baseline and the post-race data. Mean heart rate during the race (including resting time) ranged from 48 to 57% of the maximal pulse rate. Our data revealed that C1 was unaffected after the competition, while C2 decreased by 23%, but the decrease was statistically nonsinificant (p=0.09) (Table 14).

Table 12. Baseline characteristics of the study subjects

Subjects	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	VO_2max (l/min)	VO_2max/kg (ml/kg/min)
1	24	188	81.5	23.5	5.08	62.3
2	27	171	62.2	21.2	3.58	57.6
3	27	186	77.1	22.0	5.96	77.3
4	29	175	67.9	22.2	4.55	67.0
5	25	182	81.4	24.5	5.20	63.9
6	29	176	72.9	23.6	4.31	59.1
7	21	183	67.7	20.2	5.05	74.6
(x ± SD)	26.0±2.9	180.1±6.3	73.0±7.7	22.5±1.5	4.8±0.8	66.0±7.5

Table 13. Biochemical markers before and after the race

Subjects	CRP (mg/l)		Creatine kinase (mmol/l)		WBC ($x10^9 l$)	
	Before	After	Before	After	Before	After
1	1.17	7.7	282	644	5.58	4.59
2	0.62	44.3	80	542	7.50	9.33
3	1.42	1.39	424	489	4.49	3.61
4	1.05	1.91	190	313	4.24	4.25
5	1.97	2.73	253	551	7.25	8.1
6	1.18	4.34	102	539	6.95	5.72
7	0.71	0.84	165	475	6.54	4.48
(x ± SD or IQR)	1.17 (0.71; 1.42)	2.73(1.39; 7.70)*	213.7±118.1	507.6±101.6#	6.1±1.3	5.7±2.1

* p=0.03, # p=0.002 compared with the baseline values.

Descriptive analysis revealed that two cadets with VO_2max/kg >70 ml/kg/min (mean VO_2max/kg 75.95 ml/kg/min) showed a considerable increase in C1 (30%) and C2 (12%), whereas there were no remarkable changes in CRP (4.5%) after the competition. Subjects with VO_2max/kg <70 ml/kg/min (mean VO_2max/kg 62.0 ml/kg/min) revealed a significant decrease in C2 (34.3%, p=0.02) and a considerable decrease in C1 (9.7%). At the same time, among the cadets with VO_2max/kg <70 ml/kg/min CRP increased significantly (73%, p=0.04) after the competition in comparison with to the pre-race data.

Table 14. Arterial elasticity indices and MAP before and after the race

	C1 (mL/mmHg^{x}10)		C2 (mL/mmHg^{x}100)		MAP (mmHg)	
	Before	After	Before	After	Before	After
1	20.1	22.6	13.5	6.4	69.5	77.5
2	16.9	14.6	12.7	10.8	92.5	103.0
3	21.1	35.9	12.2	13.5	74.5	70.0
4	21.9	19.9	11.6	10.2	75.0	70.0
5	18.9	16.1	14.2	9.3	70.0	74.5
6	25.0	17.2	15.7	8.0	70.0	70.0
7	22.0	25.6	9.9	11.6	74.0	74.0
(x±SD)	20.8±2.6	21.7±7.3	12.8±1.9	9.9±2.3*	75.1±8.0	77.0±11.8

*p=0.09 compared with the baseline values.

5.4.2. Changes in inflammation, vascular elasticity and their associations with maximum oxygen consumption

Correlation analysis revealed that VO_2max/kg associated significantly with the difference between the baseline and the 24-hour recovery values of the small ($C2_{change}$) and large ($C1_{change}$) artery elasticity indices and with the change of creatine kinase (Figures 6 and 7). The association between VO_2max/kg and change in CRP remained statistically nonsignificant (Figure 6).

In multiple regression analysis $C2_{change}$ as the dependent variable correlated strongly with VO_2max/kg (p=0.005) and CRP_{change} (p= 0.03) (Table 15; r^2=0.89, p=0.01). At the same time, $C1_{change}$ as the dependent variable correlated only with VO_2max/kg, no significant correlation was revealed for the change of CRP.

Table 15. Multiple regression analysis for $C2_{change}$

Parameter	Regression coefficient	Standard error	p
VO_2max/kg, ml/kg/min	0.58	0.10	0.005
$CRP_{change,}$ mg/l	0.16	0.05	0.03

r^2=0.89, p=0.01.

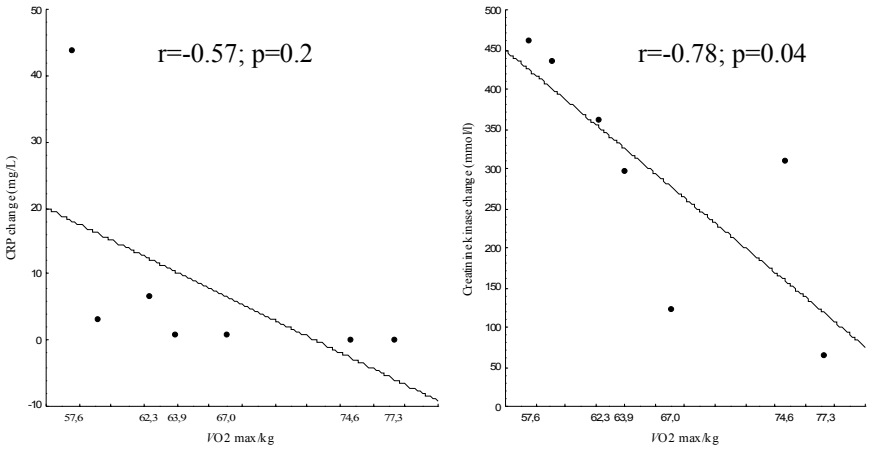

Figure 6. Correlation of VO_2max/kg either CRP $_{change}$ (left) and change in creatine kinase (right).

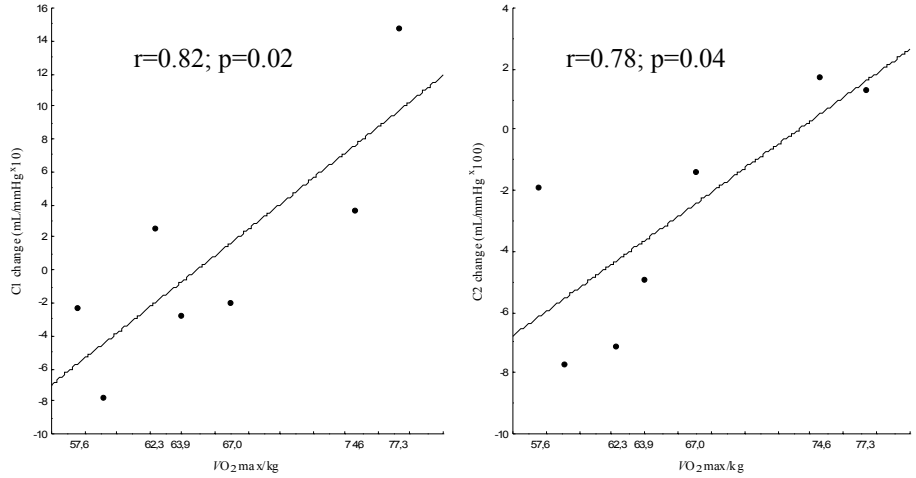

Figure 7. Correlation of VO_2max/kg with C1 $_{change}$ (left) and C2 $_{change}$ (right).

6. DISCUSSION

6.1. Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness in healthy subjects (Papers I and III)

The results of the present study showed that age, CRP, oxLDL and Hcy were differently related with arterial stiffness and IMT in healthy persons. Healthy persons with higher values of CRP had increased AIx, central PP and central SBP compared with subjects with lower values of CRP. There was independent relationship of CRP, a marker of subclinical inflammation, with AIx, a measure of arterial wave reflection and arterial stiffness. No correlation was detected between CRP and Tr, an estimated measure of aortic stiffness, or between CRP and IMT, a measure of structural changes of the large arteries. The IMT was independently correlated with oxLDL, an oxidative stress-related marker, whereas no correlations were detected between Hcy and AIx or age-adjusted IMT. In younger individuals (≤50 years) age was correlated with AIx, whereas in older individuals (>50 years) it was correlated with IMT.

Vascular ageing and arterial stiffness

Ageing and BP are the two major determinants of arterial stiffness. The effects of ageing on the proximal elastic arteries, such as the aorta and the carotid artery, are different from the effects on the distal muscular arteries. The media of the elastic arteries loses the orderly arrangement of the elastic fibers and laminae with age. Degeneration of elastic fibers is associated with an increase in collagenous material, ground substance and increase in calcification of the aortic media (Benetos *et al.* 1993). Recently, McEniery *et al.* (2005) analysed their database and demonstrated that due to age-dependent changes in the arteries, aortic PWV is more marked in older subjects (>50 years) and AIx is more prominent in younger individuals (<50 years). The investigators suggested that in younger individuals augmentation pressure rose due to an increase in the magnitude of wave reflection, whereas in older subjects the rise in augmentation pressure is driven by an earlier return of the reflected wave and a less compliant aorta. Different values of AIx or PWV in various age groups have been confirmed also by Fantin *et al.* (2007) and Mitchell *et al.* (2004). The main novel findings of Paper III were that age-related changes of AIx and IMT follow different patterns. We confirmed that AIx increases up to the age of 50 years and tends to plateau thereafter. Contrary to AIx, there was a significant increase in IMT after the age of 50 years. However, in this study we did not measure directly aortic PWV but used its estimated measure. The Tr was not related to age in older or younger subjects, as it depends to some extent also on

the magnitude of wave reflection. The results of the study confirm that in younger individuals the increase in AIx depends on the magnitude of wave reflection affected by the peripheral tone of the muscular arteries rather than by the structural changes of the proximal elastic arteries. It appears that AIx might be a more sensitive marker of arterial aging in younger individuals. However, after the age of 50 years, more structural changes were detected in the proximal elastic arteries, such as the aorta and the carotid artery, which allows IMT to become more sensitive marker of vascular aging.

Subclinical inflammation and arterial stiffness

Inflammation plays an important role in atherosclerosis (Ross 1999) and inflammatory cascade is particularly important in the atherosclerotic process. Elevated CRP has been shown to be a reliable measure of underlying subclinical inflammation and a strong predictor of future cardiovascular events (Pearson *et al.* 2003).

Paper I was the first attempt to address possible relationship between subclinical inflammation and the increased wave reflection in healthy persons. At the same time, Booth *et al*. (2004) showed independently that active systemic vasculitis is associated with increased arterial stiffness, as assessed by AIx and PWV, and that both indices were correlated with degree of active inflammation as estimated by CRP. Contrary to the results of Paper I, the same research team published data for apparently healthy individuals some months later (Yasmin *et al.* 2004b). They demonstrated that PWV was independently associated with CRP, while there was no correlation detected between AIx and CRP. This could be due to the fact that the above authors included also patients with untreated hypertension (approximately 27% of the studied subjects) and that median CRP in their patients was higher (1.9 mg/l) than in our study (0.96 mg/l). Our findings are consistent with those of Kullo *et al.* (2005), who found that CRP was significantly and independently associated with AIx and marginally with PWV (p=0.054) in asymptomatic individuals from community. By now, several studies have confirmed the relationship between PWV and CRP (Nagano *et al*. 2005; Pirro *et al*. 2004; Mattace-Raso *et al.* 2004). However some of them reported also negative results (Tomiyama *et al.* 2004; Kasayama *et al.* 2005; Kim *et al.* 2005) based on general population. Unfortunately, in all of these studies the investigators included patients with known CVD and diabetes whose average age and median CRP were significantly higher than in the subjects of our study. Different results for CRP and AIx or PWV in the above studies can probably be explained by the fact that in healthy young individuals vascular tone has a significant influence on AIx rather than on PWV (Kelly *et al.* 2001). Moreover, according to the Paper III as well as other recent reports (McEniery *et al.* 2005), age-related changes of AIx are more prominent namely in younger individuals (<50 years). On the contrary, in the case of

ageing, already established vascular disease or multiple risk factors, PWV becomes a more important determinant than AIx (Lacy *et al.* 2004).

Recently, Vlachopoulos *et al.* (2006b) published the results for 261 healthy individuals (excluding those with CVD; mean age was 41 years and median CRP was 0.89 mg/l). They found that both WBC and CRP were independently associated with AIx. The same research team confirmed these results on another healthy study group (Vlachopoulos *et al.* 2007) and demonstrated even stronger correlation between AIx and CRP than between PWV and CRP.

Our results confirm previous findings that healthy persons with higher values of CRP could represent a group at higher risk for developing cardiovascular events (Pearson *et al.* 2003). According to published categories which relate to cardiovascular risk (<1, 1 to 3 and >3 mg/l), AIx was associated with CRP in a stepwise manner in the present study.

Existing data for apparently healthy adults have demonstrated independent association between elevated CRP level and increased peripheral PP as a surrogate measure of arterial stiffness (Abramson *et al.* 2002a). The present study (Paper I) revealed no correlation between peripheral PP and CRP; correlation occurred between central PP and CRP. Several explanations can be given for the discrepancies between the above study and the present one. Firstly, in the present study CRP was measured by a high-sensitivity assay instead of standard methods for CRP. Standard assays for CRP lack the sensitivity needed to determine levels of subclinical inflammation, e.g. inflammation within a normal range (Pearson *et al.* 2003). Therefore, clinical utility for standard CRP evaluation for detection of vascular risk is extremely limited. Secondly, instead of measurement of peripheral BP, the use of noninvasive PWA in this study offers an opportunity to analyse both central and peripheral haemodynamics. As PP varies through the arterial tree, depending on the vessels´ elasticity and wave reflection, measurement of peripheral PP may not provide reliable information about central PP (Wilkinson *et al.* 2000a; Hirata *et al.* 2006), which itself defines best left ventricular workload and hence left ventricular mass – an important and independent predictor of cardiovascular mortality (Saba *et al.* 1993; Safar *et al.* 2002; Hashimoto *et al.* 2007). Thirdly, in concordance with this assumption, there has been found association of central PP with several cardiovascular risk factors as IMT (Boutouyrie *et al.* 1999) and hypercholesterolemia (Wilkinson *et al.* 2002b), whereas no such association has been found for peripheral PP.

As the present study was based on cross-sectional data, we can only speculate about the pathophysiologic mechanisms underlying the association between subclinical inflammation and arterial stiffness.

Inflammation may lead to arterial stiffening through a variety of mechanisms. CRP is not only a predictor of atherosclerotic events but is also involved in the pathogenesis of the atherosclerotic process both directly and indirectly (discussed in detail in Introduction). Cytokines may lead to increased

expression of the number of inducible enzymes that may damage the structural components of the arterial wall, e.g. metalloproteinase-9, which is capable of digesting arterial elastin. Recently positive relationship has been demonstrated between metalloproteinase-9 and arterial stiffness in apparently healthy subjects (Yasmin *et al.* 2005). Cytokines leads to an influx of inflammatory cells into the arterial wall, which *per se* may lead to arterial stiffening. Also inflammation may promote deposition of calcium within the media layer, which directly increases arterial stiffness (Abedin *et al.* 2004).

Both acute and chronic inflammation are known to impair endothelial function (Hingorani *et al.* 2000; Bacon *et al.* 2002). As patients with elevated plasma CRP levels have impaired endothelial vasoreactivity, normalisation of CRP levels over time is associated with significant improvement in endothelial dependent forearm blood flow responses (Fichtlscherer *et al.* 2000). Endothelial-derived NO is important in the functional regulation of arterial stiffness (Wilkinson *et al.* 2002a) and provides also one possible mechanism linking inflammation and arterial stiffness.

Oxidative stress and carotid artery intima-media thickness

One step in the pathogenesis of atherosclerosis is accumulation of oxLDL within plaques. These lipoproteins are considered to contribute to the inflammatory state of atherosclerosis and play an important role in its pathogenesis (Ross 1999). High plasma oxLDL concentrations might reflect its release from atheromatous plaque, which is influenced mainly by the degree of local oxidative stress, indicating that oxLDL is associated with severity of CAD and IMT (Hulthe *et al.* 2002; Vasankari *et al.* 2001). Paper III confirms the independent relationship between IMT and oxLDL. In concordance with a recent study (Elkind *et al*. 2005), we failed to show independent association between CRP and IMT in healthy persons. When the CRP subgroups were analysed, IMT increased significantly only between the first and the second subgroups. Recently, conflicting data were reported with regard to the correlation between CRP and oxLDL (Hulthe *et al.* 2002; Sjogren *et al.* 2005). The results of our study did not confirm the correlation between CRP and oxLDL. Moreover, no association was detected between oxLDL with the AIx or Tr. When the distribution of AIx in the oxLDL tertiles was analysed, a significant increase in wave reflection was detected only between the first and the second tertiles. As no correlation was detected between oxLDL and CRP, we suggest that neither marker is involved in the same pathophysiological pathway in atherogenesis. On the basis of the above findings, pathological inflammatory response might precede observable increase in oxidative stress, as was recently proposed also by Stocker and Keaney (2004). It has been demonstrated that oxLDL induces smooth muscle cell migration and proliferation by increasing expression of the platelet-derived growth factor and of the basic fibroblast growth factor (Mertens and Holvoet 2001), leading to

progression of atherosclerosis. Moreover, progressive accumulation of oxLDL within plaques itself may lead to increase of IMT.

In summary, the results of Papers I and III suggest that subclinical inflammation may affect wave reflection more than wave propagation in the large conduit arteries in healthy individuals. Systolic PWA and CRP may be more representative to describe earlier vascular changes in healthy individuals. However, with progression of atherosclerosis, more structural changes of the large arteries and increase in oxidative stress are detectable, which suggest the superiority of oxLDL and IMT. Therefore noninvasive tests for detecting early vascular changes, in combination with CRP and oxLDL, could serve as additional tools, besides the conventional cardiovascular risk factors, for assessment of cardiovascular risk, and should supplement standard risk assessment algorithms, especially in subjects at low or moderate cardiovascular risk.

Homocysteine and its associations with the functional and structural changes of arteries

Hcy has also been suggested to be an independent risk factor for CVD (Clark *et al.* 1998). Previously, weak positive associations have been demonstrated between Hcy and IMT (Voutilainen *et al.* 1998). On the contrary, Durga *et al.* (2005), did not confirm the relationship between IMT and Hcy after adjustment for sex and age. Our study showed weak correlation between IMT and Hcy, but after adjustment for age the correlation disappeared. Previously, no relationship has been reported between arterial stiffness and Hcy in general population (de Bree *et al.* 2006). Our study did not find correlation between AIx and Hcy in healthy subjects. Wilkinson *et al*. (2001b) demonstrated, moreover, that there is no effect of oral methionine loading, producing acute hyperhomocysteinemia, on AIx in healthy male subjects. Experimental data have shown that hyperhomocysteinemia may induce endothelial dysfunction via high-grade oxidative stress (Kanani *et al.* 1999). In our study no correlation was detected between Hcy and oxLDL. Moat *et al.* (2003) also reported that lowering plasma Hcy through folate supplementation was not associated with any significant change in the measures of antioxidant activity or oxidant damage in healthy individuals. Therefore the lack of association between Hcy and the structural or functional changes of the arteries in our study may be related to the fact that there were only a few patients with clinically significant hyperhomocysteinemia, which is defined as plasma Hcy >12 μmol/l and is found in 5 to 10 percent of general population (Stanger *et al*. 2003). However, the causative evidence of high homocysteine levels, leading to atherosclerosis, has been questioned in the last few years, as the discrete mechanism of vascular injury has not been identified (Darius *et al.* 2003). Also the 3 largest trials of lowering Hcy with folic acid and vitamin B12 with or without B6 (Toole *et al.* 2004; Bonaa *et al.* 2006; Lonn *et al.* 2006) consistently demonstrated no treatment

benefit for patients with established vascular disease with a slight increase in Hcy levels (<15 μmol/l). There is growing evidence that Hcy may be related to thrombosis rather than to atherosclerosis process (Caprini *et al.* 2004; Kaul *et al.* 2006).

6.2. Arterial stiffness and inflammation in patients with essential hypertension (Paper II)

The most important finding described in the Paper II was that hypertensive patients with low or moderate total cardiovascular risk had significantly increased CRP, WBC and arterial stiffness, expressed as increased AIx and Tr, compared with the sex- and age- matched control group. AIx and Tr showed a significant correlation with CRP and WBC for the whole study group. After controlling for the important confounding factors for AIx or Tr, CRP and WBC proved significant independent determinants for AIx, whereas no correlation was revealed for Tr.

Hypertension is associated with unfavorable changes in the elastic properties of the large and small arteries (Safar *et al.* 1998). During the past years an increasing body of evidence has suggested that CRP plays an important role in development of hypertension (Sesso *et al.* 2003; Sesso *et al.* 2007). Also elevated WBC is associated with the incidence of hypertension (Shankar *et al.* 2004), suggesting its pathogenic role in vascular injury (Ross 1999). The results of Paper II confirmed higher CRP levels in the hypertensive subjects compared with the healthy controls. The correlation between CRP and BP was even more significant for central BP than for peripheral BP. Moreover, arterial stiffness, measured by AIx and Tr, was increased in the hypertensive individuals in comparison with the controls. AIx and Tr showed a significant correlation with CRP for the whole study group. In the first part of the present thesis (Papers I and III) we showed that in healthy middle-age subjects inflammation affects wave reflection in the peripheral muscular arteries. In the second part of the thesis (Paper II) we included uncomplicated hypertensive subjects, without additional cardiovascular risk factors, and showed that increased BP itself or progressive inflammatory state, resulted in an increase also in large artery stiffness and wave reflection. Recently, Mahmud and Feeley (2005) confirmed the results of our study, indicating that CRP was independently associated with PWV and AIx in essential hypertension. The association between PWV and CRP in hypertensive subjects was recently demonstrated also by Kim *et al.* (2007).

It is not clear, however, whether chronic vascular inflammation is a precursor to increased arterial stiffness and hypertension, or whether high BP itself initiates inflammation and increased arterial stiffness. It has been proposed

that vascular inflammation contributes to development of stiffness rather than hypertension producing inflammation (Mahmud and Feeley 2005).

Fibrinogen is considered a marker of inflammation and a risk factor for CVD (Wilhelmsen *et al.* 1984). Engström *et al.* (2002) have demonstrated association between high plasma levels of inflammation sensitive proteins (incl. fibrinogen) and elevated BP. BP increases with the increasing number of inflammation sensitive proteins in the top quartile (>4.0 g/l for fibrinogen). In our study, no differences were found between the fibrinogen levels in the control subjects and in the hypertensive patients. Nor did multiple regression analysis reveal, after controlling for the important confounding factors for AIx, significant correlation between AIx and fibrinogen. For this several explanations can be suggested. In our study, with only three hypertensive patients with the fibrinogen value over 4 g/l, the lack of association between BP and fibrinogen is evidently due to small sample size. Comparison of fibrinogen with the other inflammatory markers (CRP or WBC) showed that fibrinogen has high intra-individual variability (de Bacquer *et al.* 1997). On the other hand, fibrinogen cannot be regarded merely as an acute phase reactant but also as an indicator of thrombotic activity (Stec *et al.* 2000). As we took great care to exclude the subjects with coronary heart disease and other cardiovascular risk factors (except for hypertension), the lack of such association was to be expected.

There are some limitations in the study. The small sample size is related to the strict exclusion criteria for the study subjects. For PWV measurement, we used its estimated measure (Tr), which depends also to some extent on the magnitude of wave reflection. Owing to the cross-sectional nature of the study, we demonstrated association between arterial stiffness and inflammation in patients with arterial hypertension but did not specify if this was the cause or the consequence of the disease.

6.3. Changes in arterial elasticity and inflammation during prolonged physical stress (Paper IV)

The major finding of Paper IV was that extreme prolonged physical load in well-trained young men caused different responses in the indices of diastolic PWA, CRP and creatine kinase depending on individual maximal oxygen consumption. A change in C2 significantly depended on VO_2max/kg and on the change of CRP, while a change in C1 was more associated with VO_2max/kg.

This study was designed to investigate the acute effect of systemic inflammation on arterial elasticity in young men. Prolonged physical stress was used as a model of acute inflammation. It is well known that intensive physical exercise is associated with acute inflammatory reaction (Liesen *et al.* 1977). Physical exercise produces microinjuries, and local inflammatory reaction in the

musculature increases CRP concentration. Several studies have suggested that regular aerobic physical training generates anti-inflammatory reaction and enhances antioxidative defense mechanisms (Criswell *et al.* 1993; Ji *et al.* 1998). Sports disciplines that are associated with excessive and long-term exertion do not always increase inflammatory reaction in the blood. It has been demonstrated that nine months of endurance training was associated with the reduction of plasma CRP in moderately trained runners (Mattusch *et al*. 1999).

Our study showed strong association between changes in C2, VO_2max/kg and changes in CRP levels, whereas a change in C1 was associated only with VO_2max/kg. Moreover, in cadets with VO_2max/kg <70 ml/kg/min physical load caused a significant increase in CRP and a decrease in C2. Interestingly, there occurred progressively inverse relationship between CRP and C2 in subjects with VO_2 max >70 ml/kg/min. The exact mechanism of the reduction in C2 in the conditions of strenuous exercise is probably multifactorial. Hence, we suggest that inflammation may have an important role in the genesis of inflammation-induced vascular dysfunction (Clapp *et al*. 2004). Experimental models of induced acute inflammation have been associated with endothelial dysfunction (Hingorani *et al.* 2000; Kharbanda *et al.* 2002). Small artery elasticity index has been shown to correlate significantly with endothelial function (Parvathaneni *et al.* 2002; Tao *et al.* 2004; Tao *et al.* 2007), reflecting at least in part endothelium-mediated arterial tone (McVeigh *et al.* 2001). In our study no changes occurred in C1, while C2 decreased in response to increased CRP. It can be suggested that in healthy young men acute inflammation affects NO mediated pathways in the smaller arteries, closer to the arterial branching points, rather than in the large arteries.

Another finding of the study is that the response of inflammatory and artery elasticity parameters to physical exercise was directly related to the subjects´ VO_2max/kg. It has been shown that endothelial function (Moyna *et al.* 2004) and arterial stiffness (Binder *et al.* 2006) are directly related to maximal aerobic capacity. It seems that higher aerobic capacity may avoid or minimize inflammation response and prevent vascular and muscular damage. It has been previously shown that regular training improves antioxidative capacity (Banerjee *et al*. 2003), decreases sympathetic tone (DeSouza *et al.* 2000) and enhances endothelial function (Clarkson *et al.* 1999). Moreover, physical activity and cardiorespiratory fitness have anti-inflammatory and antithrombotic effects that may favorably affect vascular function (Abramson and Vaccarino 2002b).

The main limitation of the study is the small number of study participants and the unusual model of acute inflammation. However, the nature of this kind of competitions is unique because of prolonged (84.5 hours) physical and psychological strain. The relatively high intensity of the long-lasting race was also described by the mean pulse rate of the study subjects (48–57% of maximal pulse rate). In the study diastolic PWA was used. The method has been subject

to a number of criticisms because of the unclear physical meaning of the model parameters and vascular arterial territory (Fogliardi *et al.* 1996; Segers *et al.* 2001). Unfortunately, due to technical reasons (the high-fidelity applanation tonometer failed at the time of this investigation), we were not able to record also systolic PWA. However, as the small number of subjects in the study did not allow to confirm with certainty the inflammation-induced impairment of C2 and its dependence on VO_2max/kg, further studies are needed to confirm our finding. This unique inflammatory model may be useful in sports disciplines that involve strenuous conditions of physical stress. The study demonstrates that it is important to monitor not only inflammatory and oxidative stress markers but also to take into account the role of individual maximal oxygen consumption and the parameters of arterial function.

7. CONCLUSIONS

1. Healthy persons with higher C-reactive protein values (>1 mg/l) had increased augmentation index, central pulse pressure and central systolic blood pressure compared with subjects with lower values of C-reactive protein. Augmentation index was independently associated with C-reactive protein. These results suggest that subclinical inflammation may be associated with arterial stiffness in asymptomatic people.

2. Carotid artery intima-media thickness was independently correlated with oxidized low-density lipoprotein in healthy subjects. This finding suggests that oxidative stress is related to structural changes in large artery wall.

3. Hypertensive patients with low or moderate total cardiovascular risk had significantly increased C-reactive protein and arterial stiffness compared with the sex- and age- matched normotensive control group. Augmentation index and timing of the reflected waveform showed significant correlation with C-reactive protein for the study group. Augmentation index was independently associated with C-reactive protein in hypertensive patients. The results confirm the evidence that increased inflammation and arterial stiffness are characteristic features of essential hypertension, and support further the link between arterial stiffness and inflammation.

4. Extreme prolonged physical load in well-trained young men caused different responses in the indices of arterial elasticity and C-reactive protein depending on individual maximal oxygen consumption. A change in small artery elasticity was significantly associated with maximal oxygen consumption and with the change in C-reactive protein, whereas a change in large artery elasticity was associated with maximal oxygen consumption only. Presence of acute inflammation produced by physical stress may play an important role in the genesis of inflammation-induced vascular dysfunction.

5. In healthy individuals younger than 50 years, age was correlated with augmentation index, whereas in healthy individuals older than 50 years, it was correlated with carotid artery intima-media thickness. The results confirm that augmentation index might be a more sensitive marker of arterial ageing in younger subjects, while carotid artery intima-media thickness plays a similar role in older subjects.

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SUMMARY IN ESTONIAN

Põletiku, oksüdatiivse stressi ja vanuse seos arteriaalse süsteemi jäikuse ning unearteri sise- ja keskkesta paksusega

Põletik on kesksel kohal ateroskleroosi patogeneesi erinevates etappides, osaledes nii endoteeli varajases kahjustuses, aterosklerootilise naastu arengus kui ka tema ebastabiilseks muutumises (Ross 1999).

Aterosklerootiliste haiguste areng sõltub põletikku vallandavate/vahendavate ja põletikuvastaste ning antioksüdantsete faktorite tasakaalust inimorganismis. Antud tasakaalu häire avaldub endoteeli kahjustuses, mis soodustab monotsüütide adhesiooni veresoone seinale ja nende tungimist subendoteliaalsesse kihti. Subendoteliaalses kihis monotsüüdid muutuvad makrofaagideks, mis neelavad sinna liikunud oksüdeeritud LDL. Rohke oksüdeeritud LDL neelamise tõttu muutuvad makrofaagid nuumrakkudeks, mis omakorda vabastavad erinevaid põletikumediaatoreid. Põletiku progresseerumine veresoone seinas avaldub süsteemses põletikulises reaktsioonis, mis omakorda soodustab monotsüütide ja oksüdeeritud LDL tungimist subendoteliaalsesse kihti, silelihasrakkude proliferatsiooni ja ekstratsellulaarse koe remodelleerimist (Zilmer *et al.* 1999; Ross 1999; Libby 2002).

Aterosklerootilise haiguse aktiivsust on kaudselt võimalik hinnata veres ringlevate ägeda faasi valkude määramisega. Erinevatest põletikumarkeritest soovitatakse subkliinilise põletiku taseme ja kardiovaskulaarse riski määramisel kasutada ultrasensitiivsel (kõrgtundlikul) meetodil määratavat C-reaktiivset valku. Viimase kohta on piisavalt läbiviidud prospektiivseid uurimusi, samuti on kättesaadav ja standardiseeritud C-reaktiivse valgu määramise metoodika (Pearson *et al*. 2003; Willerson *et al*. 2004).

Liigoksüdeerunud LDL kuhjumine veresoone subendoteliaalsesse kihti omab tugevat proaterogeennset ja põletikku vallandavat toimet (Tsimikas *et al.* 2004a). Oksüdeeritud LDL plasmaväärtused on otseselt seotud tema kuhjumisega makrofaagides ja aterosklerootilises naastus (Nishi *et al.* 2002). Oksüdeeritud LDL väärtused tsirkuleerivas veres on tõusnud aterosklerootiliste haiguste korral ja on seotud ka tulevaste kardiovaskulaarsete sündmustega (Shimada *et al.* 2004). Homotsüsteiini peetakse samuti oluliseks ateroskleroosi riskifaktoriks (Clarke *et al.* 1998), kuigi pole täpselt teada mis mehhanismiga homotsüsteiin aterosklerootilise haiguse patogeneesis osaleb. Uuringud on seostanud hüperhomotsüsteineemia aterogeenset toimet eelkõige tema võimega vallandada oksüdatiivset stressi (Virdis *et al.* 2001).

Arteriaalse süsteemi jäikus ning unearteri sise- ja keskkesta paksus on saanud tänapäeval üheks oluliseks osaks patsiendi varajase kardiovaskulaarse riski määramisel (Nichols and O'Rourke 1998; Glasser *et al.* 1997; Laurent *et al*. 2006). Uurimused on näidanud, et C-reaktiivse valgu väärtused on seotud perifeerse pulsirõhu (Abramson *et al.* 2002) ja endoteeli funktsiooniga

(Fichtlscherer *et al*. 2000), mis lubab oletada nii kroonilise kui ka ägeda põletiku mõju arteriaalse süsteemi jäikusele. Puuduvad uuringud, kus oleks näidatud seost oksüdatiivse stressi ja arteriaalse süsteemi jäikuse vahel. Vastuolulised on uuringute tulemused uudsete kardiovaskulaarsete riskifaktorite nagu C-reaktiivne valk, homotsüsteiin, oksüdeeritud LDL ja unearteri sise- ja keskkesta paksuse vahel tervetel inimestel.

Uurimuse eesmärgid

1. Uurida arteriaalse süsteemi jäikuse ja C-reaktiivse valgu vahelist seost tervetel inimestel.
2. Uurida unearteri sise- ja keskkesta paksuse ning oksüdatiivse stressi markerite vahelisi seoseid tervetel inimestel.
3. Võrrelda C-reaktiivse valgu taset ja arteriaalse süsteemi jäikust essentsiaalse hüpertensiooniga haigetel ning kontrollgrupil. Hinnata C-reaktiivse valgu ja arteriaalse süsteemi jäikuse vahelis seost antud uuringugrupis.
4. Uurida ekstreemse füüsilise koormuse mõju põletiku markeritele ning hinnata füüsilise koormusega tekitatud ägeda põletiku mõu arterite elastsusele.
5. Uurida vanuse mõju augmentatsiooniindeksile ja unearteri sise- ning keskkesta paksusele tervete uuringualuste seas.

Uuritavad ja meetodid

Uuriti 175 tervet meest ja naist vanuses 40–70 aastat, 42 kerge kuni keskmise raskusega essentsiaalse hüpertensiooniga patsienti ning 42 kontrollrühma tervet isikut vanuses 35–65 aastat. Seitset tervet meessoost sõjaväelast, vanuses 21–29 aastat, kes osalesid kolm päeva kestval võistlusel ERNA.

Kõik uuritavad läbisid tavapärase arstlikku ülevaatuse. Uuringualused, kes olid tarvitanud viimase kahe kuu jooksul regulaarselt vasoaktiivseid või põletikuvastaseid ravimeid eemaldati hilisemast uuringust. Patsiendid, kelle CRV väärtused olid üle 5 mg/l, samuti eemaldati hilisemast analüüsist, välistamaks kaasuva ägeda põletikulise haiguse võimalikku mõju.

Kerge kuni keskmise raskusastmega hüpertensiooniga patsientidel ei olnud südame isheematõve sümptomeid, hüperkolesteroleemiat, diabeeti, ega teisi olulisi kaasuvaid haiguseid. Anamneesi, läbivaatuse ja uuringute alusel välistati sekundaarse hüpertensiooni võimalus.

Terved uuringualused ja hüpertoonitõve haiged: Uuringud viidi läbi hommikul kella kaheksa ja kümne vahel tühja kõhuga. Peale 15 minutilist rahuolekus istumist mõõdeti mõlemalt õlavarrelt vererõhk, teostati süstoolse

pulsilaine analüüs, ultraheliuuring unearteri sise- ja keskkesta paksuse määramiseks ning võeti vereanalüüsid.

Sõjaväelased: Uuringud toimusid 48 tundi enne ja 24 tundi peale võistlust. Uuringud viidi läbi hommikul kella kaheksa ja kümne vahel tühja kõhuga. Peale 15 minutilist rahuolekus istumist mõõdeti patsiendi mõlemalt õlavarrelt vererõhk, teostati diastoolse pulsilaine analüüs, võeti vereanalüüsid ja teostati koormustest liikuvrajal, et hinnata maksimaalset hapnikutarbimist.

Arteriaalse süsteemi jäikus määrati süstoolse pulsilaine analüüsi meetodil (SphygmoCor Px, versioon 7.0, AtCor Medical, Austraalia). Parema käe radiaalarterilt registreeriti perifeerne rõhulaine, kasutades valideeritud ülekandefunktsiooni (Chen *et al.* 1997), tuletati tsentraalne rõhulaine aordis, millelt mõõdeti tsentraalne vererõhk, augmentatsiooniindeks ja tagasipeegeldunud rõhulaine aeg. Augmentatsiooniindeks on edaspidise ja tagasipeegeldunud rõhulaine vahe ülenevas aordis, väljendatuna protsentides pulsirõhust. Augmentatsiooniindeksi kaudu on võimalik hinnata arteriaalse süsteemi jäikust. Tagasipeegeldunud rõhulaine aeg näitab, kui kiiresti kulgeb rõhulaine ülenevast aordist suurte arterite hargnemiskohtadesse (bifurkatsioonini) ja tagasi ülenevasse aorti. Tagasipeegeldunud rõhulaine aeg hindab kaudselt aordi jäikust (Laurent *et al.* 2006).

Suurte ja väikeste arterite elastsus mõõdeti diastoolse pulsilaine analüüs abil (HDI/Pulse Wave CR-2000, Hypertension Diagnostics Inc., USA). Parema käe radiaalarterilt tonomeetriga mõõdetud pulsilaine järgi konstrueeriti suurte ja väikeste arterite elastsust peegeldavad indeksid (Chon *et al.* 1995).

Unearteri sise-ja keskkesta paksuse mõõtmisel kasutati ultraheliaparaati (LOGIQ 9, GE Medical Systems, Inglismaa) ja 12 MHz andurit. Uuringu käigus salvestati mõlemalt ühis unearterilt kolmes projektsioonis (anterolateraalses, lateraalses ja posterolateraalses) ja hilisem videosalvestuste digitaalne analüüs viidi läbi Kuopio Ülikoolis (Liu *et al.* 2004).

Uurimuse peamised tulemused

1. Käesoleva uuringu tulemused (publikatsioonid I ja III) näitasid, et tervetel uuringualustel, kelle C-reaktiivse valgu väärtused olid üle 1 mg/l, esines suurenenud augmentatsiooniindeks, tsentraalne pulsirõhk ja tsentraalne süstoolne vererõhk. Esines sõltumatu positiivne korrelatsioon C-reaktiivse valgu ja augmentatsiooniindeksi vahel. Samas puudus seos C-reaktiivse valgu ja aordi jäikuse ning unearteri sise- ja keskkesta paksuse vahel.

   Uuringu tulemustest võib järeldada, et subkliiniline põletik tervetel inimestel mõjutab enam arteriaalses süsteemis rõhulainete tagasipeegeldumist (väikestest muskulaarsetest arteritest ja arterioolidest), kui aordi jäikust ning strukturaalseid muutusi suurtes arterites.

2. Suurte arterite strukturaalseid muutusi peegeldav unearteri sise- ja keskkesta paksus korreleerus sõltumatult ainult oksüdeeritud LDLga. Puudus seos homotsüsteiini ja unearteri sise- ja keskkesta paksuse ning augmentatsiooniindeksi vahel (publikatsioon III).

   Uuringu tulemustest võib järeldada, et suurte elastsetes arterite strukturaalsete muutuste korral on suurenenud oksüdatiivse stressi tase.

3. Ravimata essentsiaalse hüpertensiooniga haigetel esines oluliselt kõrgem C-reaktiivse valgu väärtused ja suurenenud arteriaalse süsteemi jäikus võrreldes kontrollrühmaga (publikatsioon II). Augmentatsiooniindeks ja tagasipeegeldunud rõhulaine aeg korreleerusid C-reaktiivse valgu tasme ja valgevereliblede arvuga antud uuringugrupis. Mitmesel regressioonanalüüsil esines oluline seos ainult augmentatsiooniindeksi ja C-reaktiivse valgu vahel.

   Uuringu tulemused näitasid, et hüpertooniatõvega haigetel on lisaks kõrgenenud C-reaktiivse valgu tasemele ja suurenenud rõhulainete tagasipeegeldumisele kahjustunud ka aordi jäikus.

4. Tervetel treenitud meestel, kes osalesid kolm päeva kestval militaarvõistlusel ERNA, seostus arterite elastsuse langus ja C-reaktiivse valgu tõus makimaalse hapnikutarbimisega (publikatsioon IV). Väikeste arterite elastsuse muutus sõltus maksimaalsest hapnikutarbimisest ja C-reaktiivse valgu muutusest, samas suurte arterite elastsuse muutus sõltus ainult maksimaalsest hapnikutarbimisest.

   Uuringu tulemused näitasid, et äge põletik on seotud arterite funktsiooni langusega.

5. Vanus korreleerus augmentatsiooniindeksiga uuringualustel alla 50. eluaastat ja unearteri sise- ja keskkesta paksusega üle 50. eluaastat.

   Käesoleva uuringu tulemuste alusel võib järeldada, et augmentatsiooniindeks on tundlikum marker vaskulaarsete muutuste avastamisel tervetel inimestel alla 50. eluaastat, seevastu kui üle 50. eluaasta inimestel on olulisemaks vaskulaarse vananemise hindamisel unearteri sise- ja keskkesta paksuse määramine.

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PUBLICATIONS

I

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II

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. International Journal of Cardiology 2006; 112:46–51.

III

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. Journal of Hypertension 2007; 25:819–825.

IV

Kampus P, Kals J, Pihl E, Zilmer K, Pulges A, Teesalu R, Normak A, Zilmer M.
Association between arterial elasticity, C-reactive protein and maximal
oxygen consumption in well–trained cadets during three days
extreme physical load: a pilot study.
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Association between arterial elasticity, C-reactive protein and maximal oxygen consumption in well–trained cadets during three days extreme physical load: a pilot study

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ABSTRACT

Objective. Regular aerobic training has beneficial effects on inflammatory pathways and on endothelial function, which are both important cardiovascular risk factors. The aim of the present study was to evaluate the effect of extreme physical load on vascular elasticity and inflammatory markers in well-trained healthy men who participated in a high-ranking combat course.

Methods. Seven well-trained male cadets were examined during an international military combat course of 3.5-days duration. Small and large artery elasticity was assessed using diastolic pulse wave analysis. Inflammatory markers and vascular elasticity measurement were performed before and after the competition.

Results. The extreme prolonged physical load in the cadets caused a different response in arterial elasticity, C-reactive protein (CRP) and creatine kinase depending on individual maximal oxygen consumption (VO_2max/kg). VO_2max/kg correlated significantly with the change (difference between baseline and 24-hour recovery period) of creatine kinase and small and large arterial elasticity. In multivariate analysis (R^2=0.89, p=0.01) the change of small artery elasticity index correlated strongly with VO_2max/kg (p=0.005) and with the change of CRP (p=0.03). Whereas the change of large artery elasticity correlated only with VO_2max/kg, no significant correlation was revealed for the changes of CRP.

Conclusions. The major finding of the present study is that extreme prolonged physical stress in well-trained young men caused a different response in arterial elasticity, CRP and creatine kinase depending on individual maximal oxygen consumption. A change in small arterial elasticity significantly depended on VO_2max/kg and on the change of CRP, whereas the change of large artery elasticity was only associated with $VO2$ max/kg, during the strenuous 3.5-day physical load. In addition, our preliminary results indicate that acute inflammation may affect small artery elasticity.

Key words. Pulse wave analysis, arterial elasticity, inflammation, exercise

INTRODUCTION

Regular physical training reduces cardiovascular morbidity and mortality in the general population (1). It is well-known that moderate, but not mild- or high-intensity physical activity, has beneficial effects on inflammatory pathways (2) and on endothelial function (3), which are both important cardiovascular risk factors (4,5). Inflammation plays a key role in the pathogenesis of atherogenesis (6). The inflammatory process in the atherosclerotic artery leads to increased blood levels of inflammatory cytokines and other acute phase reactants. Epidemiological and clinical studies have shown strong and consistent relationship between markers of inflammation and risk for cardiovascular events (7). To date, C-reactive protein (CRP) is the most promising of these biomarkers for prediction of cardiovascular risk in terms of clinical utility (8). Research has shown that CRP is reduced significantly following nine months of endurance training in moderately trained runners (9). On the other hand, some data suggest that intense exercise may impair endothelial function through decreased levels of antioxidant capacity (10) and increased reactive oxygen species (11).

Pulse wave analysis (PWA) using a modified Windkessel model of the circulation provides a convenient noninvasive technique to assess separately large (C1) and small (C2) artery elasticity (12). Measurements of PWA are important for predicting cardiovascular risk as well as for estimating vascular abnormalities in cardiovascular disorders (12,13). Several authors have reported a link between endothelial function and C2 (14,15). Small artery elasticity may provide a possible link between endothelium-mediated arterial tone (16), indicating a relation with the functional changes in the vasculature. Thus, measurement of arterial elasticity allows us to assess the impact of prolonged strenuous exercise on vascular function (14). There is evidence that regular endurance training may have a systemic anti-inflammatory effect (9), however, there are no available data on the effect of strenuous exercise on vascular elasticity and inflammation.

The aim of this study was to evaluate the effect of extreme physical load on vascular elasticity and inflammatory markers in well-trained healthy cadets who participated in a 3.5-day international military combat course ERNA.

METHODS

Subjects and study protocol

Seven well-trained male army cadets, aged 21–29 years, were examined during an international military combat course (ERNA, Estonia) of 3.5-days duration (in total 84.5 hours). The course involved walking, jogging and special military combat activities (approximate total distance 135 km). During the race the

cadets had to avoid the reconnaissance patrol who were assigned the task of making the race as difficult as possible. The sleeping time during the race was limited to 240 minutes per night. A participant carried a backpack with special equipment and an automatic rifle with a combined weight of 25 kg. During the race food and drinks were provided as part of the equipment and additional drinks were available at each checkpoint. The covered distance and the precise location of each subject were monitored using a global positioning system (GPS). Heart rate was monitored continuously and stored at 60sec intervals using telemetry system (Sporttester Polar S810, Finland). Mean heart rate during the race (including resting time) ranged from 48–57% of the maximal pulse rate. The study protocol was approved by the Ethics Committee, University of Tartu. Informed written consent was obtained from each participant.

Forty-eight hours before the competition, all subjects passed a routine medical examination including a complete history and physical examination, electrocardiography, PWA, blood tests and exercise test on treadmill. None of the participants showed any signs or symptoms of cardiovascular disease. The subjects reported that they had not used any vasoactive, vitamin or anti-inflammatory medication during the previous two months. Blood samples were collected between 8:00 and 10:00 a.m. following an overnight fast and abstinence from tobacco, alcohol, tea or coffee, for measurements of the plasma CRP, glucose, creatine kinase, white blood cell count, red blood cell count, haematocrit, haemoglobin and platelets.

Laboratory assays

White blood cell count and red blood cell count, haematocrit, haemoglobin and platelets were measured using a Sysmex XE 2100 autoanalyser (Sysmex Corporation, Japan). CRP was determined by a validated high-sensitivity assay using a latex particle-enhanced immunoturbidimetric assay (Roche Diagnostics GmPh, Germany) with the automated analyser Hitachi 912.

Pulse wave analysis

After 15 min of rest in a supine position in a quiet temperature-controlled room, peripheral blood pressure and the arterial waveform were measured in the dominant arm by the Cardiovascular Profiling Instrument (HDI/Pulse Wave CR-2000, Hypertension Diagnostics Inc., USA). Briefly, the tonometer was applied to the patient's radial artery at the wrist overlying the radial bony prominence. The cuff for blood pressure measurement was placed on the contralateral arm and inflated concurrently with pulse waveform recording for calibration. The elasticity indices of the arteries (C1 and C2) were quantified during the diastolic portion of the cardiac cycle (mean of 30sec recording). According to the modified Windkessel model of circulation, C1 is a marker for large artery elasticity and C2 is a marker for small artery elasticity. Heart rate, mean arterial pressure and stroke volume were also calculated from the radial

pressure waveform using the HDI/Pulse Wave CR-2000 software. Haemodynamic and PWA recordings were made in duplicate. The full method has been validated and described in detail previously (17).

Incremental running exercise test

Incremental running exercise test on the treadmill was performed according to a standard protocol test using the ParvoMedics Truemax 2400 Metabolic Measurement System (ParvoMedics, USA). The subjects were required to meet two of the three standard criteria for having achieved VO_2max (heart rate ≥ age-predicted maximum heart rate, respiratory exchange ratio ≥1.10, rating of perceived exertion ≥19). Prior to testing, the gas analyser was calibrated with standard gases of known concentration.

Twenty-four hours after the competition (24-hour recovery period) all blood tests and PWA were repeated under the conditions described previously.

Statistical analysis

Statistical analysis was performed using the SPSS version 11.0. Data were expressed as the mean ± standard deviation (SD) and non-normally distributed data were presented as the median with the inter-quartile range (IQR). The data were analysed using the paired-samples *t*-test and the Wilcoxon paired test. To examine the associations between the clinical parameters, the Pearson correlation analysis and multiple regression analysis were used.

RESULTS

Unfortunately during this military competition the personal results could not be obtained due to team performance. The individual and mean values of the biochemical markers for the cadets before and after the race are presented in Table 1 and 2. There was a 58% increase in plasma creatine kinase (p=0.002) and in CRP a 57% increase (p=0.03) after the competition in comparison with baseline values. The post-race values of glucose, haemotocrit, haemoglobin, red blood cell count and platelets did not differ significantly from the baseline values (data not shown). Circulating level of white blood cell count remained unchanged after the race (Table 2). Systolic blood pressure, diastolic blood pressure and mean arterial blood pressure did not change significantly when compared with the baseline and the post-race data. Our data revealed that C1 was unaffected after the competition, while C2 decreased by 23%, but the decrease was statistically nonsinificant (p=0.09) (Table 3).

Descriptive analysis revealed that two cadets with $\dot{V}O_2$max/kg >70 ml/kg/min (mean $\dot{V}O_2$max/kg 75.95 ml/kg/min) showed a considerable increase in C1 (30%) and C2 (12%), whereas there were no remarkable changes in CRP (4.5%) after the competition. Subjects with $\dot{V}O_2$max/kg < 70 ml/kg/min (mean $\dot{V}O_2$max/kg 62.0 ml/kg/min) revealed a significant decrease in C2

(34.3%, p=0.02) and considerable decrease in C1 (9.7%). At the same time, among the cadets with VO_2max/kg < 70 ml/kg/min CRP increased significantly (73%, p=0.04) after the competition in comparison with the pre-race data.

Correlation analysis revealed that VO_2max/kg associated significantly with the difference between the baseline and 24-hour recovery values of the small ($C2_{change}$) and large ($C_{1change}$) artery elasticity indices and with the change of creatine kinase (Figures 1 and 2). The association between VO_2max/kg and change in CRP remained statistically nonsignificant (Figure 2).

In multiple regression analysis (R^2=0.89, p=0.01) $C2_{change}$ as the dependent variable correlated strongly with VO_2max/kg (p=0.005) and change of CRP (p= 0.03) (Table 4). At the same time change of large artery elasticity as the dependent variable correlated only with VO_2max/kg, no significant correlation was revealed for the change of CRP.

DISCUSSION

The major finding of the present study is that extreme prolonged physical stress in well-trained young men caused different response in indices of diastolic PWA, CRP and creatine kinase depending on individual maximal oxygen consumption. A change in small arterial elasticity significantly depended on VO_2max/kg and on the change of CRP, while the change of large artery elasticity was more associated with VO_2max/kg.

This study was designed to investigate the acute effect of systemic inflammation on arterial elasticity in young men. Prolonged physical stress was used as a model of acute inflammation. It is well known that intensive physical exercise is associated with acute inflammatory reaction (18). Physical exercise produces microinjuries, and local inflammatory reaction in the musculature increases CRP concentration. Several studies have suggested that regular aerobic physical training generates anti-inflammatory reaction and enhances antioxidative defense mechanisms (19,20). Sports disciplines that are associated with excessive and long-term exertion do not always increase inflammatory reaction in the blood. It has been demonstrated that nine months of endurance training was associated with the reduction of plasma CRP in moderately trained runners (9).

Our study showed strong association between changes in small arterial elasticity, VO_2max/kg and changes in CRP levels, whereas a change in large arterial elasticity was associated only with VO_2max/kg. Moreover, in cadets with VO_2max/kg <70 ml/kg/min physical load caused a significant increase in CRP and a decrease in C2. Interestingly, there occurred progressively inverse relationship between CRP and C2 in subjects with VO_2 max >70 ml/kg/min. The exact mechanism of the reduction in small vessel elasticity in the conditions of strenuous exercise is probably multifactorial. Hence we suggest that CRP

may have an important role in the genesis of inflammation-induced vascular dysfunction, as was recently demonstrated by Clapp et al (21). Experimental models of induced acute inflammation have been associated with endothelial dysfunction (22,23). Small artery elasticity index has been shown to correlate significantly with endothelial function (14,15), reflecting at least in part endothelium-mediated arterial tone (16). In our study no changes occurred in C1, while C2 decreased in response to increased CRP. It can be suggested that in healthy young men acute inflammation affects NO mediated pathways in the smaller arteries, closer to the arterial branching points, rather than in the large arteries.

Another finding of the study is that the response of inflammatory and artery elasticity parameters to physical exercise was directly related to the subjects´ VO_2max/kg. It has been shown that endothelial function (24) and arterial stiffness (25) are directly related to maximal aerobic capacity. It seems that higher aerobic capacity may avoid or minimize inflammation response and prevent vascular and muscular damage. It has been previously shown that regular training improves antioxidative capacity (26), decreases sympathetic tone (27) and enhances endothelial function (28). Moreover, physical activity and cardiorespiratory fitness have anti-inflammatory and antithrombotic effects that may favorably affect vascular function (29).

The main limitation of the present study is the small number of study participants. However, the nature of this kind of competitions is unique because of prolonged (84.5 hours) physical and psychological strain. The military competition presumes that participants have a high aerobic fitness level and special preparation is required for the competition. Relatively high intensity of the long-lasting race was also described by the mean pulse rate of our subjects (48–57% of maximal pulse rate). PWA is a time-consuming procedure and it has to be performed 24 hours after the race, which means that examiners had a time-dependent limitation of measurements. The small number of subjects in the study does not allow us to confirm with certainty the inflammation-induced impairment of small artery elasticity and its dependency on VO_2 max/kg. This unique inflammatory model may be useful in sports disciplines that involve strenuous conditions of physical stress. The study demonstrates that it is important to monitor not only inflammatory and oxidative stress markers but also to take into account the role of individual maximal oxygen consumption and the parameters of arterial function. The authors agree that further studies are needed to confirm our hypothesis.

In conclusion, the major finding of the present study is that severe prolonged physical and psychical strain in well-trained young men caused different responses in CRP, creatine kinase and changes in small and large artery elasticity which were directly associated with their VO_2max/kg. In addition, our preliminary results indicate that acute inflammation may affect small arterial elasticity.

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Table 1. Baseline characteristics of the study subjects

Subjects	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	VO_2max (l/min)	VO_2max/kg (ml/kg/min)
1	24	188	81.5	23.5	5.08	62.3
2	27	171	62.2	21.2	3.58	57.6
3	27	186	77.1	22.0	5.96	77.3
4	29	175	67.9	22.2	4.55	67.0
5	25	182	81.4	24.5	5.20	63.9
6	29	176	72.9	23.6	4.31	59.1
7	21	183	67.7	20.2	5.05	74.6
(x ± SD)	26.0 ± 2.9	180.1 ± 6.3	73.0 ± 7.7	22.5 ± 1.5	4.8 ± 0.8	66.0 ± 7.5

Table 2. Biochemical markers before and after the race

Subjects	CRP (mg/L)		Creatine kinase (U/L)		WBC ($x10^9$ l)	
	Before	After	Before	After	Before	After
1	1.17	7.7	282	644	5.58	4.59
2	0.62	44.3	80	542	7.50	9.33
3	1.42	1.39	424	489	4.49	3.61
4	1.05	1.91	190	313	4.24	4.25
5	1.97	2.73	253	551	7.25	8.1
6	1.18	4.34	102	539	6.95	5.72
7	0.71	0.84	165	475	6.54	4.48
(x ± SD or IQR)	1.17 (0.71; 1.42)	2.73(1.39; 7.70)*	213.7± 118.1	507.6± 101.6#	6.1± 1.3	5.7± 2.1

* p=0.03, # p=0.002 compared with the baseline values

Table 3. Arterial elasticity indices and MAP before and after the race

	C1 ($mL/mmHg^x10$)		C2 ($mL/mmHg^x100$)		MAP (mmHg)	
	Before	After	Before	After	Before	After
1	20.1	22.6	13.5	6.4	69.5	77.5
2	16.9	14.6	12.7	10.8	92.5	103.0
3	21.1	35.9	12.2	13.5	74.5	70.0
4	21.9	19.9	11.6	10.2	75.0	70.0
5	18.9	16.1	14.2	9.3	70.0	74.5
6	25.0	17.2	15.7	8.0	70.0	70.0
7	22.0	25.6	9.9	11.6	74.0	74.0
(x±SD)	20.8±2.6	21.7±7.3	12.8±1.9	9.9±2.3*	75.1±8.0	77.0±11.8

*p=0.09 compared with the baseline values

Table 4. Multiple regression analysis for $C2_{change}$

Parameter	Regression coeffficient	Standard error	p
VO_2max/kg, ml/kg/min	0.58	0.10	0.005
$CRP_{change,}$ mg/l	0.16	0.05	0.03

r^2=0.89, p=0.01

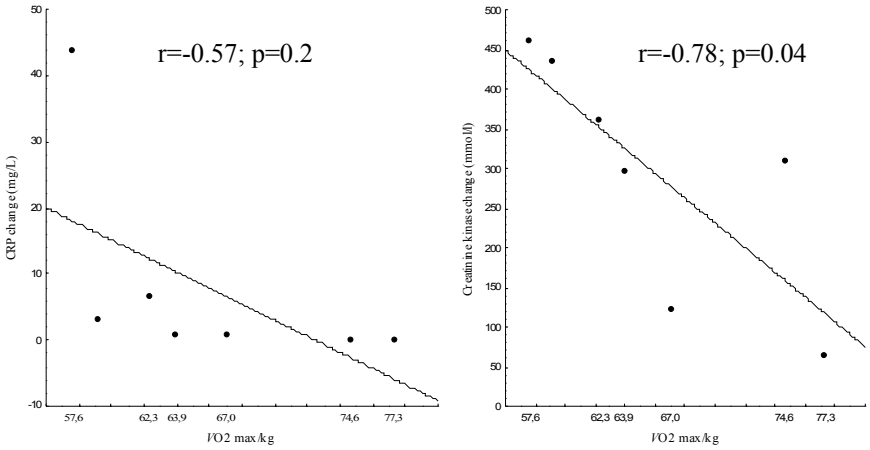

Figure 1. Correlation of VO_2max/kg either CRP_{change} (left) and change in creatine kinase (right).

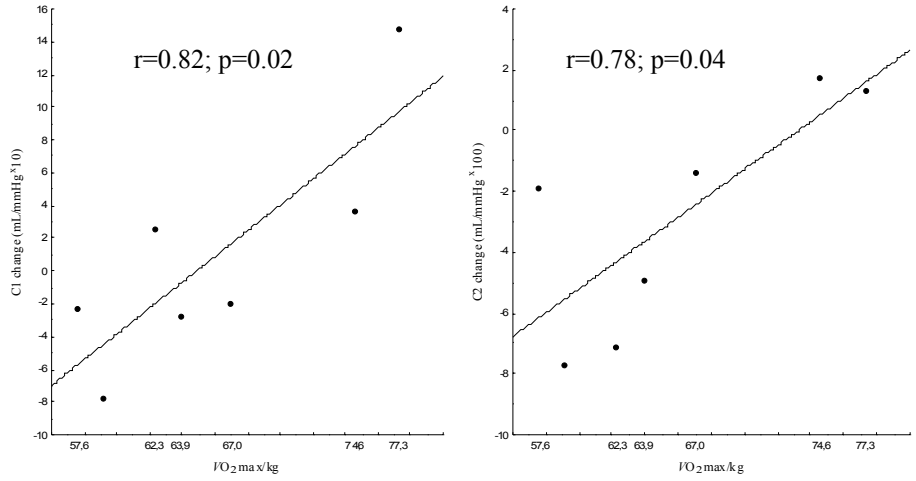

Figure 2. Correlation of VO_2max/kg with $C1_{change}$ (left) and $C2_{change}$ (right)

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Education

1992–1995	Pärnu, Secondary School No. 4
1995–2001	Faculty of Medicine, University of Tartu
2001–2002	Internship in internal medicine, University of Tartu
2002–2007	PhD studies, Department of Cardiology, University of Tartu
2005	Master of Science in Natural Science (Biomedicine) (MSc), University of Tartu
2006–	Residency in cardiology, Department of Cardiology, University of Tartu

Professional Employment

2004–	Research Fellow, Department of Cardiology, University of Tartu
2004–2007	Specialist, Department of Cardiology, University of Tartu
2005	Invited Research Fellow, Wales Heart Research Institute, University of Cardiff, Wales, United Kingdom (4 months).
2006–	Research Fellow, Department of Biochemistry, University of Tartu

Special courses

2002	European Society of Hypertension Summer School, Glasgow, UK.
2003	The Human Circulation: Noninvasive Haemodynamic, Autonomic and Vascular Monitoring. International Workshop, Graz, Austria.
2004	Theory and Applications of Pulse Wave Analysis, Vascular Research Clinic, University of Cambridge, United Kingdom.
2005	Arterial Stiffness. Theory and Practice. Vascular Research Clinic, University of Cambridge, United Kingdom.
2004	ARTERY 4. International Workshop. Royal College of Physicians, London, United Kingdom.
2005	ARTERY 5. International Workshop. Institut Oceanographique, Paris, France.

2006 Training course on arterial stiffness (organised by University of Cardiff and University of Cambridge). Budapest, Hungary.

2006 ARTERY 6. International Workshop. Athens Planetarium, Athens, Creech.

Scientific work

Main scientific research focuses upon on complex assessment of arterial function in humans as well as in animal models, and to investigate the role of inflammation and oxidative stress in vascular dysfunction.

21 scientific publications (including 11 articles in international peer-review journals) and 19 presentations at international scientific conferences.

Membership: Board member (treasurer) of the Estonian Society of Hypertension, associate Editor in the journal "*Vererõhk*" (Blood Pressure – in Estonian), member of the Estonian Society of Cardiology, member of the Estonian Society of Young Doctors.

Publications in peer-review journals

1. 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. The Scandinavian Journal of Clinical and Laboratory Investigation 2007 (in press)
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11. Kals J, Kampus P, Põder P, Pulges A, Teesalu R, Zilmer M. Impaired endothelial function in patients with peripheral arterial disease. Proceedings of the 5th International Congress on Coronary Artery Disease 2003; 351–354.
12. Kals J, Kampus P, Pulges A, Zilmer M, Teesalu R. Ülevaade kaltsiumikanali antagonistide toimest endoteelile. Eesti Arst 2003; 82:834–838.
13. Kals J, Kampus P, Põder P, Pulges A, Teesalu R, Zilmer M. Pulsilaine analüüs – uudne mitteinvasiivne meetod endoteeli funktsiooni hindamiseks. Eesti Arst 2003; 82:423–430.
14. Kampus P, Kals J, Ristimäe T, Zilmer K, Zilmer M, Teesalu R. Projekt "Atero-geensuse Staatuse Skriinimine Eesti Rahvastikul" (ASSER): Uudne käsitlusviis südame-ja veresoonkonnahaiguste preventatsioonis. Eesti Arst 2003; 82:29–34.
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Haridus

1992–1995	Pärnu Ülejõe Gümnaasium
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2001–2002	Tartu Ülikooli arstiteaduskonna üldinternatuur
2002–2007	Tartu Ülikooli arstiteaduskonna doktorantuur
2005	Biomeditsiini magister (MSc), Tartu Ülikool
2006–	Tartu Ülikooli kardioloogia eriala residentuur

Erialane teenistuskäik

2004–	Tartu Ülikooli kardioloogiakliiniku teadur
2004–2007	Tartu Ülikooli kardioloogiakliiniku spetsialist
2005	Cardiffi Ülikooli külalisteadur (Inglismaa) (4 kuud)
2006–	Tartu Ülikooli biokeemia instituudi teadur

Erialane täiendus

2002	Euroopa Hüpertensiooni Ühingu suvekool. Glasgow Ülikool, Inglismaa.
2003	Rahvusvaheline *work-shop*: The Human Circulation: Noninvasive Haemodynamic, Autonomic and Vascular Monitoring. Karl-Frenzese nimeline Grazi Ülikool. Graz, Austria.
2004	Rahvusvaheline seminar: Pulsilaine analüüs. Vaskulaaruuringute Keskus, Cambridge Ülikool, Inglismaa.
2004	Rahvusvaheline *work-shop*: ARTERY 4. Royal College of Physicians, London, Inglismaa.
2005	Rahvusvaheline seminar: Pulsilaine analüüs. Vaskulaaruuringute Keskus, Cambridge Ülikool, Inglismaa.
2005	Rahvusvaheline *work-shop*: ARTERY 5. Pariis, Prantsusmaa.
2006	Arteriaalse süsteemi jäikus ja hüpertensioon. Cardiffi ja Cambridge Ülikooli vaskulaaruurijate talveseminar. Budapest, Ungari.
2006	Rahvusvaheline *work-shop*: ARTERY 6. Ateena, Kreeka.

Teadustegevus

Minu teadustöö põhi suundadeks on arterite funktsiooni (arteriaalse süsteemi jäikuse, endoteeli funktsiooni, arterite strukturaalsed muutused) ja uudsete vaskulaarset kahjustust peegeldavate biomarkerite uurimine ning nende rakendamine kliinilises praktikas. Töötame välja uudseid biokeemiliste markerite ja instrumentaalsete parameetrite paketti, mida saaks rakendada südame- ja veresoonkonna haiguste varajases preventatsioonis.

Ilmunud on 21 teaduspublikatsiooni (neist 11 rahvusvahelistes eelretsenseeritavates ajakirjades), 19 ettekannet rahvusvahelistel konverentsidel.

Eesti Hüpertensiooni Ühingu juhatuse liige (laekur), arteriaalse hüpertensiooni Eesti ravijuhise töögrupi liige, ajakirja "Vererõhk" toimetuskolleegiumi liige, Eesti Kardioloogide Seltsi liige, Eesti südame- ja veresoonkonna haiguste preventatsiooni ravijuhise töögrupi liige, Eesti Nooremarstide Ühenduse liige.

Artiklid eelretsenseeritavates ajakirjades

1. 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. The Scandinavian Journal of Clinical and Laboratory Investigation 2007 (in press)
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