Inflammation and Lifestyle in Cardiovascular Medicine

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“If you have an apple and I have an apple and we exchange these apples then you and I will still each have one apple. But if you have an idea and I have an idea and we exchange these ideas, then each of us will have two ideas.”

George Bernard Shaw
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ABSTRACT

Despite major advances in the treatment and prevention of atherosclerosis the last several decades, cardiovascular disease still accounts for the majority of deaths in Sweden. With the population getting older, more obese and with rising numbers of diabetics, the cardiovascular disease burden may increase further in the future.

The focus in cardiovascular disease has shifted with time from calcification and narrowing of arteries to the biological processes within the atherosclerotic plaque. C-reactive protein (CRP) has emerged as one of many proteins that reflect a low grade systemic inflammation and is suitable for analysis as it is more stable and easily measured than most other inflammatory markers. Several large prospective studies have shown that CRP is not only an inflammatory marker, but even a predictive marker for cardiovascular disease. C-reactive protein is associated with several other risk factors for cardiovascular disease including obesity and the metabolic syndrome.

Our study of twenty healthy men during a two week endurance cross country skiing tour demonstrated a decline in already low baseline CRP levels immediately after the tour and six weeks later.

In a study of 200 obese individuals with impaired glucose tolerance randomised to a counselling session at their health care centre or a one month stay at a wellness centre, we found decreased levels of CRP in subjects admitted to the wellness centre. The effect remained at one, but not after three years of follow-up.

In a prospective, nested, case-referent study with 308 ischemic strokes, 61 intracerebral haemorrhages and 735 matched referents, CRP was associated with ischemic stroke in both uni- and multivariate analyses. No association was found with intracerebral haemorrhages. When classifying ischemic stroke according to TOAST criteria, CRP was associated with small vessel disease. The CRP 1444 (CC/CT vs. TT) polymorphism was associated with plasma levels of CRP, but neither with ischemic stroke nor with intracerebral haemorrhage.

A study on 129 patients with atrial fibrillation was used to evaluate whether inflammation sensitive fibrinolytic variables adjusted for CRP could predict recurrence of atrial fibrillation after electrical cardioversion. In multivariate
models, lower PAI-1 mass was associated with sinus rhythm even after adjusting for CRP and markers of the metabolic syndrome.

In conclusion, lifestyle intervention can be used to reduce CRP levels, but it remains a challenge to maintain this effect. CRP is a marker of ischemic stroke, but there are no significant associations between the CRP1444 polymorphism and any stroke subtype, suggesting that the CRP relationship with ischemic stroke is not causal. The fibrinolytic variable, PAI-1, is associated with the risk of recurrence of atrial fibrillation after electrical cardioversion after adjustment for CRP. Our findings suggest a pathophysiological link between atrial fibrillation and PAI-1, but the relation to inflammation remains unclear.

Key words: C-reactive protein, cardiovascular disease, stroke, atrial fibrillation, lifestyle, Interleukin-6, Tumor necrosis factor-α, exercise, physical activity, obesity, the metabolic syndrome, fibrinolysis.
Trots stora framsteg i behandling och prevention av hjärt- och kärlsjukdomar de senaste decennierna svarar de fortfarande för merparten av dödsfallen i Sverige. Med en allt äldre befolkning med ökande andel överviktiga och diabetiker kan den kardiovaskulära sjukdomsbördan komma att öka ytterligare i framtiden.

Fokus för studierna av hjärt-kärlsjukdom har med tiden skiftat från förkalkning och förträngning av artärer till biologiska processer i det aterosklerotiska placket. C-reaktivt protein (CRP) är ett av många proteiner som återspeglar en läggradig systemisk inflammation, lämpligt för analys eftersom det är stabiler och lättare att mäta än de flesta andra inflammatoriska markörer. Flera stora prospektiva studier har visat att CRP, förutom att vara en inflammatorisk markör, också är en markör för kardiovaskulära sjukdom. C-reaktivt protein är förknippat med flera andra riskfaktorer för hjärt- kärlsjukdomar inklusive fetma och det metabola syndromet.

I en studie av två friska män under en två veckor lång fjälltur på skidor visade vi att deras redan i utgångsläget låga CRP sjönk ytterligare efter turen och sex veckor efter att turen avslutats var CRP fortfarande lägre än före studiens start.


I en studie med 308 hjärninfarkter, 61 hjärnblödnings och 735 matchade kontroller var CRP associerat med insjuknande i hjärninfarkt men inget samband kunde påvisades med hjärnblödning. Vid klassificering av ischemisk stroke enligt TOAST kriterier var CRP associerat med hjärninfarkt orsakad av småkärlsjuka. En genetisk variant av CRP gav upphov till högre CRP nivåer utan något samband påvisades med varken hjärninfarkt eller hjärnblödning.

En studie av 129 patienter med förövareffler har använts för att analysera om inflammationskänsliga fibrinolyssvariabler justerat för CRP kunde förutsäga återfall i förövareffler efter elkonvertering. Lågre PAI-1 var
associerat med sinusrytm även efter justering för CRP och det metabola syndromet.

Sammanfattningsvis kan livsstilsintervention användas för att sänka CRP nivåer men det är en utmaning att bibehålla den nya hälsosammare livsstilen. CRP är en markör för hjärnfarkt men det finns inget samband mellan de genetiska varianter i CRP genen som orsakar förhöja CRP nivåer och stroke. Detta talar mot att det skulle vara förhöjda nivåer av CRP som orsakar stroke. Även efter korrigerande för CRP är lågt PAI-1 är associerat med bibehållen sinusrytm efter elkonvertering. Våra fynd talar för patofysiologiska samband mellan förmaksflimmer och PAI-1 men relationen till inflammation är fortfarande oklart.
ABBREVIATIONS

AF    Atrial fibrillation
BMI   Body mass index
CI    Confidence interval
CRP   C-reactive protein
CVD   Cardiovascular disease
HDL   High density lipoprotein cholesterol
LDL   Low density lipoproteins
IIG   Intensive intervention group
IL-6  Interleukin 6
MCP-1 Monocyte chemoattractant protein-1
MMPs  Metalloproteinases
MONICA MONItoring trends and determinants in CArdiovascular disease
OGTT  Oral glucose tolerance test
OR    Odds ratios
PAI-1 Plasminogen activator inhibitor-1
RCT   Randomised controlled trials
SNP   Single-nucleotide polymorphism
TNF-α Tumor necrosis factor-α
TOAST Trial of Org 10172 in Acute Stroke Treatment
t-PA  Tissue plasminogen activator
UCG   Usual care group
VCAM-1 Vascular cell adhesion molecule-1
VIP   Västerbotten Intervention Programme
ORIGINAL PAPERS

This thesis is based on the following papers:


IV. Andersson J, et al. Markers of fibrinolysis and vWF as predictors for recurrence of atrial fibrillation after electrical cardioversion. Accepted for publication in *Thrombosis Research* 2010.
PREFACE

When I began my medical studies I found the hospital environment unpleasant and thought I would never work in such a place. Later I found the surroundings changing and becoming rather peaceful, but it could have been me. In my clinical practice I realized that it was this that I wanted to do and the hallways of the research department remained a shadowy environment I would never visit. When I came to Skellefteå Hospital this, too, changed but again, it could have been me. Whether it is me or my surroundings that change, I do believe that the change is a result of inspiring colleagues and treasured friends around me.
INTRODUCTION

Cardiovascular disease today

The most common cause of death in Sweden can be referred to diseases from the circulatory system, mainly the heart and blood vessels, which account for over forty percent of all deaths. These include coronary heart diseases, congestive heart failure, cerebrovascular diseases, hypertension, cardiac arrhythmias and peripheral arterial disease among others. They are usually summarized as cardiovascular disease (CVD).

Even though the mortality from CVD has demonstrated an astonishing decrease in Sweden since 1987 to 2007 CVD remains the most common cause of death. Sweden shares this impact of CVD on health with the developed world making this disease an immense and global challenge. Cardiovascular disease is an epidemic which through epidemiological transition now represents the leading cause of death in the world with approximately 80% of these deaths occurring in low and middle income countries.

The atherosclerotic plaque - biological processes

The main cause of CVD is considered to be atherosclerosis which develops in the intima, the inner lining of the blood vessel formed by the endothelial cells of large and medium-sized elastic and muscular arteries, figure 1.

A response to injury caused by smoking, hypertension, diabetes, hypercholesterolemia, disturbed blood flow or a combination of these and other factors lead to dysfunction of the endothelial cells. In this setting, low density lipoproteins (LDL) depositions in the intima undergo modification and oxidizes unless removed by another type of cholesterol transporter, high density lipoproteins.

To remove the accumulating oxidized LDL, pro-inflammatory leukocyte adhesion molecules such as P-selectin and vascular cell adhesion molecule-1 (VCAM-1) are expressed on the surface of the endothelial cells to attract monocytes.

As monocytes migrate into the intima stimulated by the cytokine monocyte chemoattractant protein-1 (MCP-1) they change characteristics and become
macrophages. Macrophages have inflammatory properties, ingest oxidized LDL through expression of scavenger receptors forming foam cells and eventually die, developments which propagate the inflammatory process.\textsuperscript{5} This early and reversible stage of atherosclerosis called fatty streaks consisting of lipid rich macrophages and T lymphocytes has been observed already during youth.\textsuperscript{3}

Figure 1
A. Fibrous cap of smooth muscle cells and collagen.
B. Lipid core with oxidized LDL, macrophages, foam cells and necrotic cells.

If the damage to the artery continues, smooth muscle cells from the vessels media layer migrate into the subendothelial intima and proliferate.\textsuperscript{6} Connective and elastic tissue, inflammatory cells, cholesterol and calcium constitute a matrix that finally forms the atheroma or atherosclerotic plaque.\textsuperscript{7} As the atheroma grows larger the artery dilates, a process called remodelling, partly compensating for reduced lumen size.
The migration and proliferation of vascular smooth muscle cells and collagen-rich matrix deposition creates a fibrous cap surrounding the atheroma. If growing large enough, the atheroma may bulge into the artery and reduce blood flow and, in worst case, rupture. However, even non-obstructive plaque may rupture with subsequent thrombosis that in turn obstructs blood flow.
Atherosclerosis – an inflammatory process

Our understanding of the development of atherosclerosis has changed from that of hypercholesterolemia and stenosed arteries to a much more complex picture. Many patients with myocardial infarction have normal cholesterol levels and plaque rupture may depend more on the characteristics of the fibrous cap and atheroma than the degree of obstruction of the artery.\textsuperscript{3, 6}

When looking back at the described development of an atherosclerotic plaque we observe that it involves inflammatory processes at all stages, figure 2. The main risk factors for arteriosclerosis are related to inflammation. Smoking, hypertension, obesity, diabetes and the metabolic syndrome are all associated with a low grade inflammation.\textsuperscript{8}

Endothelium

Oxidation of LDL results in local inflammation contributing to endothelial expression of the adhesion molecules P-selectin and VCAM-1 that recruit lymphocytes and monocytes from the blood stream to the endothelium.\textsuperscript{9}

Macrophages

Macrophages have several inflammatory properties. They are antigen-presenting cells and express scavenger receptors. They also secrete cytokines such as tumor necrosis factor-\( \alpha \), chemokines, growth-regulating molecules such as platelet-derived growth factor, metalloproteinases (MMPs) and other hydrolytic enzymes upholding an inflammatory state and adding to plaque progression and instability of the fibrous cap.\textsuperscript{3}

Smooth muscle cells

Endothelial cells, together with macrophages and smooth muscle cells are the major inflammatory cells of atherosclerosis. The smooth muscle cell is a major producer of extracellular matrix in the vessel wall and can express receptors for lipid uptake. They also express adhesion molecules such as VCAM-1 and intercellular adhesion molecule-1 to attract monocytes and lymphocytes to the vessel wall. Finally, they produce cytokines such as platelet derived growth factor and MCP-1 which propagate inflammation.\textsuperscript{6, 10}

The matrix of the atheroma also contains several other inflammatory cells that contribute to plaque development. T-lymphocytes attracted to the lesion through VCAM-1 become activated by macrophages and dendritic cells
differentiating to cytokine producing and pro-inflammatory Th-1 helper cells or to Th-2 cells with more anti-inflammatory properties.\textsuperscript{11}

Mast cells are also found in the atheroma and can produce proteinases that activate MMPs, destabilising the fibrous cap. They produce both TNF-α and histamine, the latter increasing vascular permeability facilitating leukocyte recruitment.\textsuperscript{4}

The discovery of inflammatory processes during development of the atherosclerotic plaque have led to the idea of atherosclerosis as an inflammatory disease, bringing advancements to our understanding of atherosclerosis and providing new ways to improve risk assessments and to develop new treatment regimes.

Figure 2
Inflammatory processes during atherosclerosis.
C-reactive protein

The most studied protein in the context of inflammation and CVD is undoubtedly C-reactive protein (CRP). CRP, discovered in 1930 by William Tillet and Thomas Francis at the Rockefeller Institute, was identified as a substance in serum that reacted with the C polysaccharide of pneumococcus. It became the first acute-phase protein to be described and is a non-specific systemic marker of infection, inflammation, tissue damage (i.e. necrosis, trauma) and malignancy. CRP is recognized to activate the complement system through the classical pathway.

CRP is a member of the pentraxin family of calcium-dependent ligand-binding plasma proteins with a constant half-life of about 19 hours. The only determinant of CRP concentration is thus its rate of synthesis. CRP is produced in the liver mediated via interleukin 6 and enhanced by interleukin-1 and TNF-α. The median concentration of CRP is about 0.8 mg/l with a 90th centile at 3.0 mg/l and 99th centile 10 mg/l in adult volunteer blood donors aged 18-63 years. There is a considerable within-subject variation of CRP suggesting that three serial measurements of C-reactive protein, as for blood pressure in the diagnosis of hypertension, should be done to achieve reliability comparable to total cholesterol. Smoking, obesity and hormone replacement therapy increase CRP levels, whereas moderate alcohol consumption and statins lower CRP levels. Levels of CRP are substantially genetically determined, up to 40 percent. Ethnicity and age also influence CRP levels. In a study of different European populations, median CRP values up to 44 years of age were 0.6–1.1 mg/L and 1.2–1.7 mg/L among those 45 years and older. Levels were found to be similar in men and women. In another larger study, women had higher levels of CRP than men. The reason for the diverging results may depend on the exclusion of women on contraceptives and hormone replacement therapy, which increase CRP levels, in the first but not the latter study.

CRP in atherogenesis

The first indication of CRP involvement in atherogenesis came in 1982 when de Beer showed that CRP binds selectively to LDL molecules, a finding that was later confirmed for oxidized LDL. Macrophages use the scavenger receptor for uptake of oxidized LDL molecules, but uptake of native LDL has been demonstrated only when opsonized with CRP.

CRP is chemotactic for human blood monocytes and this may be mediated through inducing adhesion molecule expression in human endothelial cells.
and increased MCP-1 levels. CRP downregulates endothelial NO production, the main determinant of basal vessel tone, and induces monocytes to synthesize tissue factor, an important pro-coagulant. Furthermore, CRP induces plasminogen activator inhibitor type 1 (PAI-1) expression in endothelial cells which have implications for atherothrombosis. Many endothelial cell activation studies concerning the effects of CRP in vitro are, however, disputed as possible artefacts caused by azide and lipopolysaccharide contaminations found in commercial CRP solutions making results difficult to interpret.

Studies on human coronary arteries show that CRP deposition precedes the appearance of monocytes in early atherosclerotic lesions. One in vivo study on hypercholesterolemic rabbits demonstrated a close relationship between the CRP plasma levels and atherosclerosis. CRP seems to originate from the circulation rather than synthesized by macrophages as suggested by previous studies on CRP solutions. That mRNA for CRP has been found in human plaques also suggests a vascular CRP production. Furthermore, CRP is present in human coronary plaques, especially in ruptured fibrous caps. Suggestions of extrahepatic CRP production in atherosclerotic plaques are also supported from findings of a CRP-gradient between coronary arterial blood sampled just distal and proximal to human coronary plaques, a gradient that was higher in unstable than stable angina suggesting that it could be a potential marker of plaque instability. C-reactive protein induces matrix metalloproteinases that may contribute to this plaque instability.

**Interleukin-6**

Interleukin-6 (IL-6) is produced by a variety of cells including endothelial cells, monocytes/macrophages and mast cells and production is induced by viral infections or cytokins as interleukin-1, TNF-α and interferon-γ. It can act as growth factor and is involved in T-cell activation. In fatty tissue, IL-6 is produced under stimulation by TNF-α and high IL-6 levels in patients with the metabolic syndrome are associated with truncal fat mass. Contracting skeletal muscle, dependent of exercise duration, intensity and muscle mass involved, induces IL-6 production in muscle tissue, even without muscle damage. The half-life of IL-6 is short, less than two hours.

An important feature of IL-6 is its role as a primary inducer of the acute phase response leading to inflammation in response to tissue damage due to trauma, malignant tumors or infection. It stimulates production of the acute phase proteins, among them CRP. Interleukin-6 increases rapidly after
infection, inducing fever and releases adrenocorticotropic hormone as a part of the acute phase response.\textsuperscript{39}

Anti-inflammatory properties are also described for IL-6. It stimulates production of the anti-inflammatory interleukins IL-1ra and IL-10 and inhibits production of TNF-\(\alpha\).\textsuperscript{40} Finally, IL-6 induces lipolysis among other metabolic properties, possibly to regulate metabolism during exercise, but its role in the metabolic syndrome remains controversial.\textsuperscript{40}

**Tumor necrosis factor-\(\alpha\)**

Tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) was discovered in 1968 and named for its ability to cause tumor necrosis through an anti-tumoral response of the immune system.\textsuperscript{42} TNF-\(\alpha\) is a cytokine and acute phase protein that intercorrelate with IL-6 and CRP. TNF-\(\alpha\) stimulates IL-6 production which in turn inhibits TNF-\(\alpha\). Plasma levels of TNF-\(\alpha\) increase with age.\textsuperscript{40} Primarily produced by macrophages, TNF-\(\alpha\) is also produced by lymphoid cells, mast cells, endothelial cells, fibroblasts and neuronal tissue.\textsuperscript{43} In addition to acting as a major proinflammatory mediator it is also able to induce apoptosis and augment several autoimmune diseases as rheumatoid arthritis and inflammatory bowel disease.\textsuperscript{43}

TNF-\(\alpha\) is important for the innate immune system and is released in response to lipopolysaccharides and other bacterial substances. It seems most important for defence against some intracellular pathogens and is considered an important cytokine in development of septic shock. Contrary to the high concentrations that promote shock, prolonged exposure to low concentrations cause cachexia as can be seen among tumor patients.\textsuperscript{43}

Increasing data suggest that TNF-\(\alpha\) is involved in the metabolic syndrome. TNF-\(\alpha\) is produced in adipose tissue, induces lipolysis and is involved in insulin signalling and sensitivity.\textsuperscript{40}

**Atrial fibrillation**

Atrial fibrillation is the most common sustained arrhythmia causing uncoordinated atrial activation and deterioration of atrial mechanical function. This can often, but not always, be observed by the patient as a fast and irregular heart rhythm and is usually diagnosed through ECG examination. Atrial fibrillation may occur in presence or absence of structural heart disease. Atrial fibrillation increases the risk of thromboembolic events and stroke.\textsuperscript{44} These are some factors explaining the
increased morbidity and mortality associated with atrial fibrillation. In addition to stroke, atrial fibrillation is also associated with all cause mortality and heart failure.\textsuperscript{44} The cause of atrial fibrillation is not fully understood, but there are several hypotheses including atrial electrical remodelling, focal causes, atrial ischemia and dilation, age-related structural changes and even inflammation.\textsuperscript{44, 45} Increased PAI-1 mass concentration and its complex with tissue plasminogen activator levels have been described in atrial fibrillation.\textsuperscript{46-48} PAI-1 has also been found to predict recurrence of atrial fibrillation.\textsuperscript{49, 50} Endothelial dysfunction and metabolic pathways may contribute to the inflammatory and hypofibrinolytic state in atrial fibrillation.\textsuperscript{50-52}

**Risk factors of cardiovascular disease – established risk factors**

Prevention of cardiovascular disease is the primary objective for both patients and health care providers and requires knowledge on how disease can be prevented. In 1948, one of the most important public health studies for establishing risk factors of cardiovascular disease was initiated, the Framingham Heart Study. They early identified hypertension, obesity and hypercholesterolemia as conditions associated with atherosclerotic heart disease, risk factors that today remains of great importance.\textsuperscript{53}

Since then, through the subsequent works with the Framingham risk factors and other epidemiological studies, these associations have been confirmed and further risk factors identified.

The INTERHEART Study from 2004 showed that eight risk factors accounted for the majority of the modifiable risk for cardiovascular disease in both sexes and at all ages. These were high blood lipids, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, low consumption of fruits, vegetables, and alcohol, and lack of regular physical activity.\textsuperscript{54} The subsequent INTERSTROKE Study showed that the same risk factors were equally important in the prediction of the risk of both ischemic and hemorrhagic stroke.\textsuperscript{55}

Increasing age, male gender and heredity are also known risk factors for CVD. They are pre-determined and cannot be changed and are therefore often called non-modifiable risk factors to separate them from those factors, which if improved, will reduce the risk of CVD. Still, they are important for determining risk of developing CVD so as to emphasize the importance of taking corrective measures on modifiable factors.
Concept of risk factor versus risk marker

A risk factor could be an environmental, behavioural or biologic factor that is statistically associated with an outcome. A generally accepted definition of a risk factor in medicine does not exist, but in practice the previously mentioned modifiable risk factors such as hypertension and hypercholesterolemia have generally been established in large randomised controlled trials (RCT) where removal or reduction of the factor has rendered a decline in cardiovascular mortality. The golden standard for defining risk factors is therefore considered a large RCT.

In clinical practice we prefer to identify factors which after intervention modify the risk of an event. With this as a backdrop, Beck has suggested a practical definition of risk factors as “an environmental, behavioral, or biologic factor confirmed by temporal sequence, usually in longitudinal studies, which if present, directly increases the probability of a disease occurring, and if absent or removed, reduces the probability. Risk factors are part of the causal chain, or expose the host to the causal chain. Once disease occurs, removal of a risk factor may not result in a cure.”

Those factors that are associated with an increased probability of a pre-specified outcome without a causative relation are sometimes referred to as risk markers, figure 3.
Figure 3
Differences between risk factor and risk marker.
Causality

Even though a risk factor must show a temporal sequence so that the cause precedes the effect, it does not imply that it is part of a causal chain. We must bear in mind that there are no tests of significance that can prove causality. The RCT is great for defining a risk factor according to Beck, but do notice that he writes that a risk factor does not necessarily have to be causal; it may only “expose the host to the causal chain”.

The question of causality is hence separate from that of a risk factor. Sir Bradford Hill, English epidemiologist and statistician who pioneered the randomized clinical trial, suggested in 1965 nine viewpoints from which all association should be studied before the question of causation can be determined. None of his viewpoints were to be considered indisputable evidence. In the end, the question of causation was, and remains, a judgement.

Bradford Hill’s viewpoints

1. The strength of the association is of importance as a stronger association suggests causality.
2. Consistency of an observed association strengthens a possible causality as the association then is found by different persons, in different places, circumstances and times.
3. Specificity suggests causality if the association is limited to specific conditions as specific environment, situation and disease.
4. Temporality, the cause must precede the effect and if a delay between the cause and effect is expected the effect must occur after such a delay.
5. Biological gradient, the finding of a dose-response curve suggests causality.
6. It is preferable if there is biological plausibility to causation, but we cannot demand plausibility as it is limited to current knowledge.
7. Coherence is present if the association merge with or at least not contradict generally known facts of the disease.
8. Experiment, when appropriate, may give clues to causation. The randomized controlled trial is one example.
9. At last analogy may suggest causation. Hill refers to how the knowledge of thalidomide and rubella would make us prone to accept “slighter but similar evidence with another drug or another viral disease in pregnancy”.

Almost 50 years after publication of what is now known as Hill’s criteria it still remains an important principle for deciding causality. Bradford himself
referred to them as “viewpoints” as he considered none of them required or enough for evidence of causation.

**Inflammation as predictor of cardiovascular disease**

In the early eighties, studies showed a peak of CRP levels about 50 hours after the onset of myocardial infarction which was thought to be a response to tissue damage.\(^5\) Gradually the theory of inflammation as a part of the development of atherosclerosis, and not only a response to tissue damage, arose. The first prospective studies on CRP as a predictor for coronary heart disease came in 1996\(^5\) and 1997.\(^6\)

Of the many inflammatory markers available CRP is considered most suited as a marker of systemic low-grade inflammation due to various analytic and assay characteristics as summarized by a statement by American Heart Association.\(^6\) There seem to be little seasonal or diurnal variation with CRP. It is stable, has acceptable within-individual variation, and several reliable assays are available. There is also a World Health Organization standard.\(^6\)

Furthermore, but with minor importance, CRP is well-known among clinicians.

In the late 1990’s a relationship between CRP and CVD was observed in several large, high quality studies in different populations which persisted after adjustment for established risk factors. Meta-analyses on well conducted studies have confirmed CRP as an independent risk marker for CVD including coronary heart disease, ischemic stroke and total vascular mortality.\(^23, 62-66\)

C-reactive protein is not, in the context of inflammation, exclusive as predictor for future CVD. Several other inflammatory variables have been studied as risk markers for cardiovascular disease. For example, TNF-α, IL-6\(^6\), fibrinogen\(^6\), P-selectin and adhesion molecules such as VCAM-1 are all predictors of cardiovascular disease.\(^6\) This may indicate that the specific source of inflammation may not be important for the cardiovascular risk.

**CRP and cardiovascular disease – the question of causality**

Currently there is an ongoing debate as for whether CRP is a risk marker or a risk factor. From epidemiological studies CRP is a well established risk maker for CVD, but yet no RCT with a CRP inhibitor has been conducted. When looking at Hill’s viewpoints we find that CRP is robustly and consistently associated with CVD. It shows temporality and a biological gradient; higher levels of CRP are associated with an increased risk of CVD.
It is biologically plausible that CRP could increase inflammation and atherosclerosis, a theory that does not contradict current knowledge. However, the specificity of CRP can be debated as it is associated with several established risk factors for CVD and other markers of inflammation are also predictive for CVD.\(^8\), \(^71\)

Basic science research using Mendelian randomization have been conducted.\(^72\), \(^73\) As mentioned earlier, CRP levels are to a high degree genetically determined. Mendelian randomization uses nature’s random assortment of genes causing different basal CRP levels to assess the causal nature of CRP as a risk factor for CVD. This could be compared to a randomized controlled trial of genetic variants. Both Zacho\(^75\) and Elliot\(^74\) have found CRP levels to be dependent on a number of polymorphisms. These polymorphisms were clearly associated with CRP levels. However, the polymorphisms leading to higher CRP levels did not translate into an increased risk of CVD. This argues against a causal association and makes it unlikely that a RCT with a CRP antagonist would reduce CVD.

The interpretation of Mendelian randomization studies requires caution. It is not an experimental setting as is the case for a RCT. In Mendelian randomization one studies an intermediate phenotype or gene product that is influenced by the genotype. Gene-environment interactions or developmental adaptation to a genetically elevated CRP level can exist influencing results from these studies.\(^76\)

Even so, these and other studies suggest a non-causative relationship between CRP and CVD. This does not diminish the importance of inflammation in CVD, remembering that the use of CRP as a measure of low-grade systemic inflammation was initially a quite practical choice. Inflammation may still be important in CVD pathogenesis and regardless of causality CRP remains a risk marker for CVD that can improve risk assessment.\(^62\), \(^77\)

**Inflammation and physical activity**

Moderate physical activity for 30 minutes at least five times per week is an important, well-recognized, and encouraged method to reduce the risk of cardiovascular disease.\(^78\), \(^79\) The mechanisms behind the beneficial effects of physical activity on cardiovascular health is yet unresolved, but we know that the amount of leisure-time physical activity is inversely associated with CRP and IL-6 levels.\(^80\)-\(^82\) Higher levels of IL-6, CRP, and fibrinogen are independently associated with lower maximal oxygen consumption\(^83\) and exercise training decreases systemic inflammation.\(^82\), \(^84\)
It may also be that physical inactivity itself causes inflammation. Physical inactivity is associated with elevated levels of IL-6 and CRP independent of age, gender, smoking and obesity.\textsuperscript{85}

Strenuous physical exercise as running a marathon causes haemostatic changes and an acute phase response with increased levels of CRP, D-dimer, fibrinolytic activity and von Willebrand factor while fibrinogen levels decrease.\textsuperscript{86, 87} The inflammatory response during strenuous physical exercise seems to be mediated by the cytokine system, mainly IL-6, and is of short duration compared to lower sustained levels of inflammatory markers seen in long term physical training and among more physically active individuals.\textsuperscript{82} It is still unclear how physical exercise suppresses CRP levels, but both weight loss and decreases in IL-6 levels have been suggested as mediators.\textsuperscript{88, 89} The knowledge of the effects of physical activity on inflammation in both the short and long term among healthy, non-athletic individuals is also limited.

In conclusion, physical activity decreases both the systemic inflammation and the risk of cardiovascular disease; the mechanism behind this remains unanswered. Whether the cardiovascular health benefits of physical activity is mediated through inflammation or not is an important subject for future research.

**Inflammation and the metabolic syndrome**

Increased CRP levels in obesity are at least partially mediated through IL-6 derived from adipose tissue.\textsuperscript{90} Elevated CRP levels are found predominantly among those obese who are also insulin resistant and levels decrease with weight loss-associated improvements in insulin resistance. The relation between CRP concentrations and insulin resistance also seem to be independent of obesity.\textsuperscript{93, 92} Independent of lifestyle or surgical intervention, CRP levels decrease approximately 0.13 mg/L per 1 kg of weight loss.\textsuperscript{93} The largest benefit from weight reduction on CRP levels are found in obese individuals with insulin resistance.\textsuperscript{91}

The metabolic syndrome consists of increased waist circumference, fasting glucose, blood pressure, triglycerides and decreased high density lipoprotein cholesterol (HDL).\textsuperscript{94, 95} Inflammation as measured by CRP is associated to each and every component of the metabolic syndrome\textsuperscript{96} and CRP levels increase with the number of components of the metabolic syndrome.\textsuperscript{71, 97} BMI alone\textsuperscript{98} or central obesity together with hypertension have been suggested as the main determinants for elevated CRP levels in the metabolic syndrome.\textsuperscript{99}
In addition to CRP, IL-6 and TNF-α are associated with total and central obesity and fibrinolytic variables. Subjects with the metabolic syndrome also have higher levels of fibrinogen and white blood cell counts suggesting an inflammatory component in the metabolic syndrome.

In prospective studies, CRP, IL-6 and TNF-α have been found to predict development of type 2 diabetes, adjusting for BMI did not affect results on IL-6 but TNF-α was not longer significant. For CRP the association remained significant after adjusting for BMI in one of two studies.

**Coagulation and fibrinolysis**

Coagulation is the transformation of fibrinogen to fibrin creating a blood clot to stop bleeding; however it may also cause thrombosis formation within vessels thereby causing tissue damage. The haemostatic equilibrium maintains a balance between the coagulation and its opposite, fibrinolysis, figure 4.

![Diagram of Coagulation and Fibrinolysis](#)

Figure 4
Coagulation and fibrinolysis.
- activation
- PAI-1 inhibits t-PA by forming a complex
Thrombin converts fibrinogen to its active form that creates a fibrin clot. Thrombin is formed after a process initiated by the binding of factor VII to a cofactor called tissue factor. Thrombin can also activate the anticoagulant protein C system with thrombomodulin as a cofactor or, less efficiently, by thrombin alone.

Fibrinolysis is the process whereby the fibrin clot is broken down. The main enzyme in fibrinolysis is plasmin which degrades the fibrin mesh. Plasmin is the activated form of plasminogen. Tissue plasminogen activator (t-PA) converts plasminogen to active plasmin. t-PA is released into the blood by damaged vascular endothelium. t-PA is in turn inhibited by plasminogen activator inhibitor-1 (PAI-1), forming an inactive tPA/PAI-1 complex. The balance between coagulation and fibrinolysis is called haemostasis.

**Inflammation and haemostasis**

There is an association not only between CRP and body mass index (BMI), waist circumference, blood pressure, insulin levels, HDL and triglycerides but also to plasminogen activator inhibitor-1 antigen and tissue-type plasminogen activator antigen. It is well known that acute inflammation, in particular in combination with sepsis, may cause a systemic activation of the coagulation system called disseminated intravascular coagulation. The cytokine, IL-6, seems to be a major mediator for the activation of coagulation in DIC and the protein C system may modulate the outcome of severe inflammation.

There is support for the involvement of inflammatory cytokines in coagulation which can contribute to thrombosis formation. CRP has pro-coagulant effects by induction of PAI-1 expression and activity in endothelial cells and tissue factor production in monocytes. PAI-1 is found in several tissues, probably mainly produced by the liver, but also in adipocytes and acts as an acute-phase reactant. Fibrinogen in the coagulation system also acts as an acute phase reactant and is a predictor of CVD as previously described. TNF-α induces expression of tissue factor from monocytes and decreases expression of thrombomodulin in vitro. In obesity several cytokines, among them TNF-α, can stimulates the production of PAI-1 in adipose tissue.

Coagulation can also affect inflammatory markers. Thrombin has been shown to induce a variety of non-coagulant effects, among them production of MCP-1 and IL-6 in fibroblasts and mononuclear cells in vitro.
mentioned, thrombin can also activate the protein C system which has anti-inflammatory properties.\textsuperscript{107}

In conclusion, there is a complex relationship between inflammatory and fibrinolytic variables, both in infectious and a cardiovascular context. Infection has been suggested as causative to CVD and that it may explain increased CRP levels.\textsuperscript{119} Our knowledge is limited in this subject but it is clear that inflammation can shift the haemostatic balance.

**Inflammation, haemostasis and the metabolic syndrome**

Adipose tissue have been suggested to contribute to the prothrombotic state observed in obesity due to its ability to enhance coagulation by increasing synthesis of coagulation factors such as fibrinogen and tissue factor and by increasing PAI-1, TNF-\(\alpha\), and IL-6, thereby affecting inflammation and haemostasis.\textsuperscript{51, 117} The number of components of the metabolic syndrome also correlate with levels of fibrinogen, IL-6 and white cell count.\textsuperscript{71}

Our knowledge on the interactions between components of the metabolic syndrome, inflammation and haemostasis is increasing. Whether the associations between circulating inflammatory markers levels are independent of obesity and whether inflammation is the cause of insulin resistance or a consequence of obesity remains to be answered.\textsuperscript{120} Haemostasis is a potential mediator of increased cardiovascular risk in both the metabolic syndrome and among those with increased CRP levels, but this subject needs further investigation. A possible relation between inflammation, the metabolic syndrome, haemostasis and physical activity is shown schematically in figure 5.

In the coming decades we may begin to identify high risk individuals for type 2 diabetes through CRP assessment on order to intensify preventive actions. Such strategies might target inflammation itself via well known methods as diet and exercise. Increased knowledge of the role of inflammation in obesity and development of diabetes type 2 may also improve present strategies and lead to new preventive treatments.
Inflammation is related to several other markers of cardiovascular disease. The strength, independence, mechanisms and importance of these relations needs better understanding and may lead to improvement of cardiovascular health through better risk stratification and treatment options.
OBJECTIVES

The objective of this thesis was to improve the knowledge of the relationship between lifestyle and inflammation among healthy and diseased subjects and to examine the utility of inflammatory markers as predictors for cardiovascular disease.

The specific aims of the papers were:

I) To explore the effects of endurance physical activity on CRP, IL-6 and TNF-α during and after heavy physical exercise and to study if diet modifies the inflammatory response during endurance physical activity.

II) To study the effects of lifestyle intervention on CRP levels among obese individuals with impaired glucose tolerance.

III) To study CRP as a marker of intracerebral hemorrhage and ischemic stroke, both total and subclassified by ischemic stroke type, using TOAST criteria. To study the relationship between the 1444C>T polymorphism, plasma levels of CRP and stroke.

IV) To explore the role of activity and mass concentrations of PAI-1 and t-PA as predictors of maintenance of sinus rhythm 30 days after electrical cardioversion with adjustment for inflammatory and metabolic factors.
MATERIALS AND METHODS

Paper I

Study population and design

Explanatory post-hoc analysis within a randomized intervention study of twenty fit, but non-athletic men, aged 18-55, who participated in a 14 days cross-country skiing tour. The daily trips varied between 12 and 30 km corresponding to exposure of about ten hours of heavy physical activity defined by cross-country skiing, digging snow caves and carrying backpacks weighing about 25-30 kg. The participants were randomized to a diet with either 40 or 30 energy percent from fat, but with no limitation regarding energy intake.

A few months before the igloo-tour each participant registered all consumption of food and beverages during seven days in a food diary. For this purpose special registration forms and instructions including pictures of portion sizes, elaborated by the Swedish National Food Administration, were used. Based on these seven-day records the individual intakes of energy and nutrients were calculated by computer at the National Food Administration. In the analyses only data on the intake of energy, protein, fat, carbohydrates, dietary fibre and polyunsaturated/saturated fat-ratio of the diet were used. Four weeks after the end of the two week long igloo-tour, at week six, the participants were provided with a two week ration of the same foods as they had consumed during the tour, but adapted in such a way that the daily energy intake was the same as the average daily energy intake during the week before the tour, as defined by the food diary. While on this diet blood samples were drawn at the end of week eight. Body weight and height were measured after an overnight fast and BMI was calculated. Blood samplings were taken at the start of the tour (baseline), after the first and second week of exercise and in the recovery period at weeks six and eight from baseline, figure 6.
Figure 6
Study design of paper I.
Paper II

Study population and design

This was a randomized controlled trial on subjects with a BMI $>27$ kg/m$^2$ and impaired glucose tolerance. Between the years 1985 and 1994, participants from the Västerbotten Intervention Programme (see paper III) with a normal fasting glucose value were offered an oral glucose tolerance test (OGTT). An invitation was sent out by mail to the 650 individuals who fulfilled the inclusion criteria defined as an abnormal OGTT and a BMI $>27$ kg/m$^2$; 345 individuals expressed interest in participation and accepted the randomized design of the study. 41 subjects who had already participated in a lifestyle modification programme and three subjects who were too ill to participate were excluded. Of the remaining 301 subjects, 100 randomized to the intensive intervention group (IIG) and 100 to the usual care group (UCG). The remaining subjects ($n=101$) were assigned as substitutes. Twenty substitutes were enrolled in the IIG and eight in the UCG. Another six participants withdrew their participation from the UCG before the study start, but for logistical reasons they were not replaced by substitutes, figure 7.

Intervention

The participants in the IIG were admitted for a one month stay at a wellness centre. Baseline was set to the time of admittance to the wellness centre. A physical examination with blood sampling and an OGTT were conducted after an overnight fast on the first or second morning from admittance. The programme included approximately 140 h of scheduled activities including exercise of low to moderate intensity daily for 2.5 h (e.g. brisk walks, gymnastics, cycling and swimming). The diet served contained approximately 20% of energy from fat and a relatively high fibre content. Recommended portion sizes were calculated to approximately 7.6 MJ (1800 Kcal) for men and 6.3 MJ (1500 Kcal) for women, leading to a slow but persistent weight decline. Alcoholic beverages were not allowed and smoking cessation was strongly encouraged. Furthermore, health-promoting coping strategies together with stress management and relapse-prevention techniques were emphasized. The participants were encouraged to make plans on how to incorporate healthy lifestyle changes in everyday life. The examination protocol, together with additional learning sessions, was repeated during a 4-day stay at follow-up 12 months later. A phone call inquiring about the status of the lifestyle change was placed to the participants in the IIG at six months and at two years from the study start.
Figure 7
Study design of paper II.
Participants randomized to the UCG underwent a health survey with a physical examination, an OGTT and blood sampling at their health-care centre or by their general practitioner at baseline. The survey ended with a counselling session for 30-60 min conducted by a specially trained nurse. The participants were given both oral and written advice focusing on lifestyle intervention towards impaired glucose tolerance and obesity. The protocol and a new short counselling session were repeated at follow-up after 12 months.

Similar follow up visits, including a health examination and blood sampling, were carried out at 3 and 5 years in both study groups. A power calculation showed that 99 participants in each group with a standard deviation of 5.0 mg/L would detect a decrease in CRP from 3 mg/L to 1 mg/L at a power of 80%. As CRP levels above 3 mg/L are associated with higher cardiovascular risk, such a decrease would potentially be of clinical importance.
Paper III

The northern Sweden cohorts

This was a prospective, population-based, case-referent study within the northern Sweden cohorts which consisted of the Västerbotten Intervention Programme (VIP) and the WHO MONItoring trends and determinants in CArdiovascular disease (MONICA) study in northern Sweden. 74,000 individuals participated in the Northern Sweden Cohorts from 1985 to 1999. Participants were requested to donate blood samples and were asked to answer a questionnaire with items on social background, smoking habits, medical history, and drug intake.

The WHO MONICA study in northern Sweden

The WHO Monica study was initiated in 1982 in 26 countries with the objective of measuring trends in cardiovascular disease and mortality. In the framework of MONICA, population-based surveys were performed in 1986, 1990, 1994 and 1999 in Norrbotten and Västerbotten counties with a total population of about 510 000. In 1986 and 1990, a sample of 2000 individuals aged 25-64 years were randomly selected and invited (stratified for age and sex) to participate. In 1994 and 1999 the age span was increased to 25-74 years and the sample to 2500 individuals. The participation rate in the MONICA study in northern Sweden was over 75%.

The Västerbotten Intervention Programme

The Västerbotten Intervention Programme started in 1985 as a community intervention program on CVD and diabetes prevention in the county of Västerbotten, Sweden. All men and women upon reaching 30, 40, 50 and 60 years of age in Västerbotten county are invited to participate in a screening program for CVD and diabetes and requested to answer a questionnaire at their local primary health care centre (since 1996 the individuals reaching 30 years are no longer included). Participants undergo an examination and health counselling focusing on lifestyle and risk factors for CVD and diabetes. All parts of the intervention, questionnaire, examination and blood sampling were designed to be as similar to the MONICA survey as possible. The participation rates have ranged between 48 and 67 %.
Case and referent definition

For the purpose of identifying of first-ever stroke cases, hospital records, general practitioners reports and death certificates were screened for stroke events and validated according to the MONICA criteria. The WHO MONICA criteria excluded transient ischemic attacks, subdural hemorrhage, and strokes with concomitant brain tumour or severe blood disease. Cases with definite first-ever stroke were defined by the Northern Sweden MONICA Incidence Registry during the period of January 1, 1985, to September 20, 2000. Stroke subtypes were divided into intracerebral hemorrhage (International Classification of Diseases, ninth revision (ICD-9 code 431), cerebral infarction (ICD-9 code 434), and unspecified stroke (ICD-9 code 436) see figure 8. Cases diagnosed as subarachnoid hemorrhages were excluded. Intracerebral hemorrhage was diagnosed through a positive finding on CT scan and/or autopsy. For cerebral infarction, no sign of hemorrhage on CT scan or autopsy was allowed. Cerebral infarction cases were further divided into large vessel disease, cardioembolic stroke, small-vessel disease, other specified and undetermined stroke according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. A computerized algorithm was used for defining TOAST subtypes.

Two referents for each case were randomly selected from the same population-based health surveys. Referents were matched for sex, age (±2 years), date of health survey (±1 year), type of survey (VIP or MONICA), and geographic region. Referents were also excluded if they had died or moved out of the MONICA region before the date of the index event.
74,000 individuals participated in the Northern Sweden Cohorts from 1985 to 1999.

582 stroke cases identified January 1, 1985, to September 20, 2000.

70 cases excluded due to previous myocardial infarction, 42 due to previous cancer and 84 due to insufficient blood samples. Six cases of unspecified stroke (ICD-9 code 436) and 11 cases with missing CRP analysis were also excluded.


Figure 8
Population in paper III.
Paper IV

Study population and design

We conducted a pre-specified hypothesis generating study within a double-blinded, placebo-controlled, randomized, prospective, investigator-initiated multicentre study of the effects of atorvastatin on recurrence of atrial fibrillation (AF) after cardioversion. Between August 2004 and January 2007, a total of 234 patients with persistent AF and an indication for cardioversion were included. Five of the participating centres collected blood samples from their 129 included patients for the present study. Persistent AF was defined as AF with duration of more than 7 days. Patients under 18 years and over 80 years of age were excluded, as were patients with paroxysmal AF, atrial flutter, contraindications to atorvastatin, ongoing treatment with lipid-lowering drugs, ongoing treatment with class I or class III antiarrhythmic treatment, oral amiodarone six months before inclusion, or known liver disease or myopathy, as well as patients with a previous electrical cardioversion during the last 12 month. Treatment with beta receptor-blocking agents, calcium antagonists, diuretics, digoxin, ACE-inhibitors, ARBs, and acetylsalicylic acid was used when clinically indicated. Before randomization, i.e. at baseline, a detailed medical history was obtained and a transthoracic echocardiographic examination, 12-lead electrocardiography, and basic laboratory analyses were performed including PAI-1 activity, PAI-1 mass, tPA activity, tPA mass, vWF, CRP and blood lipids. Patients were then randomized to 80 mg Atorvastatin or placebo, initiated at least 14 days before the elective cardioversion. Further samples and follow-up were made at day 2 and 30 days after cardioversion, figure 9.

Figure 9
Study design in paper IV.
### Populations

Table 1 shows a compilation of baseline variables in papers I-IV for comparison of the study populations. In paper IV, there was no measurement of blood pressure. For paper I there is no data on smoking. For Paper II and referents in Paper III, age at screening is shown. For ischemic strokes, age refers to age at the stroke event.

<table>
<thead>
<tr>
<th></th>
<th>Paper I All participants</th>
<th>Paper II Ischemic stroke</th>
<th>Paper III Referents</th>
<th>Paper IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>n=20</td>
<td>n=193</td>
<td>n=308</td>
<td>n=735</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36 (30; 45)</td>
<td>60 (50; 60)</td>
<td>61 (54; 66)</td>
<td>60 (50; 60)</td>
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<tr>
<td>Sex M/F (% men)</td>
<td>20/0 (100)</td>
<td>70/122 (36)</td>
<td>175/133 (57)</td>
<td>436/209 (59)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>-</td>
<td>16 (8)</td>
<td>73 (24)</td>
<td>141 (19)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 (66; 86)</td>
<td>84 (77; 92)</td>
<td>77 (67; 87)</td>
<td>76 (66; 83)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22 (21; 27)</td>
<td>30 (29; 32)</td>
<td>26 (24; 29)</td>
<td>26 (24; 28)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.4 (0.4; 4.2)</td>
<td>2.4 (1.3; 5.1)</td>
<td>1.4 (0.7; 3.1)</td>
<td>1.0 (0.5; 2.2)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>120 (111; 125)</td>
<td>140 (126/154)</td>
<td>143 (129; 154)</td>
<td>133 (122; 146)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70 (65; 80)</td>
<td>85 (80; 92)</td>
<td>88 (83; 94)</td>
<td>83 (79; 91)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.0 (4.5; 5.7)</td>
<td>5.6 (5.0; 6.3)</td>
<td>6.3 (5.6; 7.1)</td>
<td>6.2 (5.3; 6.9)</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.3 (2.9; 3.9)</td>
<td>3.6 (3.1; 4.2)</td>
<td>4.3 (3.8; 5.0)</td>
<td>4.2 (3.6; 5.0)</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.3 (1.2; 1.5)</td>
<td>1.2 (1.0; 1.4)</td>
<td>1.1 (1.0; 1.5)</td>
<td>1.3 (1.0; 1.6)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.7 (0.6; 1.0)</td>
<td>1.8 (1.3; 2.5)</td>
<td>1.5 (1.0; 2.1)</td>
<td>1.3 (0.9; 1.8)</td>
</tr>
</tbody>
</table>

Table 1
Compilation of baseline variables in paper I-IV. Median and 25th and 75th percentiles are given.
Laboratory procedures in study I-IV

Plasma samples for lipid measurement were obtained after a minimum of 4 hours of fasting. In Papers I and II, total cholesterol and triglycerides were analyzed using kits from Roche/Boehringer (Mannheim, FRG) and LDL and HDL cholesterol were measured by direct, homogeneous assays based on detergent treatment of the serum or plasma (N-geneous™ HDL-c and N-geneous™ LDL reagents, respectively, from Genzyme Corporation, Cambridge, MA, USA). Cholesterol was measured with Reflotron bench-top analysers in the Västerbotten Intervention Program, and by an enzymatic method (Boehringer Mannheim GmbH, Germany) at a central laboratory in MONICA. Plasma CRP was determined with an automated high sensitive C-reactive protein method (IMMULITE, Diagnostic Products Corporation, USA), the interassay coefficient of variation was <6%. IL-6 and TNFα were also analysed using IMMULITE and the inter-assay coefficient of variation was 5.1% for IL-6 and 6.5% for TNFα. For analysis of fibrinolytic markers, blood samples were collected in Stabilyte tubes and analysis was carried out using an enzyme-linked immunosorbent assay (ELISA). Chromolize PAI-1 and Chromolize t-PA from Biopool AB, Sweden, was used to determine activities of PAI-1 and tPA, respectively. Tintelize PAI-1 and Tintelize t-PA was used to determine mass concentrations of PAI-1 and tPA, respectively.

Genotyping for the CRP 1444C>T SNP (rs1130864) was performed by a 5´-nuclease (TaqMan) assay on an ABI PRISM 7900HT sequence detector system (Applied Biosystems) as described. A subset (10% of the samples) was reanalyzed and all results were consistent.

The terms t-PA and PAI-1 mass are often named t-PA and PAI-1 antigen and includes t-PA and PAI-1 in complexes as shown in figure 10. In paper IV, the terms t-PA mass and PAI-1 mass are used. Antigen should be separated from t-PA and PAI-1 activity which are t-PA and PAI-1 in uncomplexed (active) form.
Figure 10
Schematic representation of the relationship between t-PA antigen and activity, PAI-1 antigen and activity and t-PA/PAI-1 complex.
Statistical analyses

All analysis was performed using Statistical Package for the Social Sciences (SPSS) version 11.5.1 (Chicago, IL, USA) for Papers I-III and version 17.0 for Paper IV. Two-tailed tests were performed and P-values below 0.05 were considered as statistically significant.

Paper I

Median and 25th and 75th percentiles were calculated for baseline variables. Mann-Whitney U test was used to study differences between the two dietary groups and Wilcoxon’s rank sum test for comparison within the groups during the course of the study. Spearman rank-order correlation coefficient and linear regression models were used to examine the associations between variables.

Paper II

Mann-Whitney U test was used for comparisons between the two treatments groups and Wilcoxon’s signed ranks for comparison within the groups. In linear regression analysis the dependent variable CRP was log-transformed. Relation between changes in CRP and changes in body weight, glucose and insulin were analysed with linear regression. Differences between groups of categorical variables were calculated using Chi-square tests. Analysis was made according to intention to treat.

Paper III

The distribution of CRP was skewed and presented as geometric means. Spearman rank-order correlation coefficient was used to examine the correlation between the studied variables. To test the relation between CRP and the risk of stroke, CRP was categorized into three risk groups <1mg/L, 1-3mg/L, and >3mg/L. Conditional logistic regression analyses were used to calculate odds ratios (OR) and 95% confidence interval (CI). Multivariate logistic regression was performed to estimate the effects on different risk markers when controlling for continuous (BMI, total cholesterol) and categorical (CRP, smoking, hypertension and diabetes) variables. Deviation from Hardy-Weinberg equilibrium was tested by a χ² test. Wilcoxon rank sum test was performed to test genotype-phenotype associations. A power calculation showed that an OR of <0.65 and >1.49 for CRP could be detected at a power of 80% with a significance level of 5% from our 311 cases of
ischemic stroke. Corresponding values for the 61 cases of intracerebral hemorrhage were <0.34 and >2.43.

**Paper IV**

The Mann-Whitney U test was used for comparisons between groups and the Wilcoxon signed ranks test for comparisons within the groups. Chi-square was used for grouped variables when comparing the subgroup with the entire population. Correlation between variables was tested with Spearman’s test. Logistic regression was used to calculate the odds ratio and 95% CI for components of the fibrinolytic system to predict rhythm at 30 days while adjusting for potential confounding factors. In the initial model fibrinolytic variables were analysed, thereafter the contribution of CRP was analysed. The components of the metabolic syndrome (hypertension, triglycerides, BMI and cholesterol) were then added and, finally, age and smoking.
Etical considerations

The study protocols for study I to III were approved by the Research Ethics Committee of Umeå University and data handling procedures were approved by the National Computer Data Inspection Board when appropriate. Study IV was approved by the Ethics Committee at Karolinska Institute and the Swedish Medical Product Agency. All studies complied with the Declaration of Helsinki. Informed consent was obtained from patients in all studies. For study III, all subjects were informed at the time of the health examination and gave written consent to future use of examination data and blood analyses for research purposes. Data analyses were consistently performed on anonymous data sets so no individuals could be identified.
RESULTS

Effect of physical activity on CRP, IL-6 and TNFα. (Paper I)

The study base was twenty normotensive men with a normal BMI, aged 18-55 years. Intense physical exercise was performed during the first two weeks of the study with a six week follow-up period.

Median CRP level was 1.37 mg/L at baseline and increased significantly during the first week of exercise to 5.0 mg/L (p=0.00) and then decreased significantly during the second week to 2.0 mg/L (p=0.02). From baseline to the end of the study CRP decreased significantly to 0.7 mg/L (p=0.02).

CRP levels did not correlate to weight or LDL, nor did the change in CRP correlate to the change in weight or LDL from baseline to the end of study. In a linear regression model, CRP was associated with IL-6, but not with TNFα, lipids, or weight at baseline and the change of CRP during the study was associated with the change in IL-6 (p=0.018).

There was no change in IL-6 from baseline to the end of the study. From the second week of exercise, where IL-6 levels peaked, to the end of study, there was a significant decrease in IL-6 levels (p=0.01). Regression analysis showed that change in IL-6 during the study was associated with changes in CRP and TNFα.

TNFα increased significantly from baseline to week one (median levels from 6.8 pg/ml to 7.9 pg/ml; p=0.03) and two (median level 8.4 pg/ml; p=0.00). There was no change in TNFα between baseline and end of the study. As for IL-6, the decrease from the second week to the end of the study was significant. The TNFα levels were not associated with lipids.

Weight decreased significantly after two weeks of exercise and remained significantly lower at the end of study. We analysed correlations between CRP, IL-6, TNFα, BMI and weight. CRP was correlated to IL-6 at baseline (r=0.702 p=0.001) and week one of exercise (r=0.484 p=0.03), but not to any other variable at any time. IL-6 and TNFα did not correlate to each other or to BMI or weight at any time.
Dietary effects on inflammation during physical activity

We did not observe any significant difference between the two dietary groups in the inflammatory variables, weight, BMI, age, systolic and diastolic blood pressure at any time during the study. Significant changes in CRP levels were only seen within the dietary group with 40 energy percent from fat where it increased during the first week, but was lower between baseline and end of the study.

TNFα increased significantly in both dietary groups after two weeks of exercise. Thereafter levels dropped significantly to the eighth week. There was no difference in TNFα levels in either group from baseline to the end of the study. For IL-6 there were no significant changes within either group.
Effect of lifestyle intervention on CRP in obese with impaired glucose tolerance. (Paper II)

The study population was 193 subjects with an abnormal OGTT and a BMI >27 kg/m². At baseline the intervention group had a higher body mass index, CRP levels and lower triglycerides and fasting plasma insulin levels than the control group. CRP levels during the study are presented in figure 11.

Smoking, higher BMI and female sex were correlated to a higher CRP level at baseline when studying all participants.

No difference in CRP levels was found at one year follow-up between the two treatment groups. There was a significant reduction in CRP within the intervention group after one month at a wellness centre. This decrease persisted at the one year follow up, but not after three years. When comparing the change in the CRP level from baseline to the one year follow up between the groups there was a significant reduction of the CRP level in the intervention group compared with the control group.

To study how the change in CRP related to the changes in metabolic parameters, we analysed the change in these parameters from baseline to the one month of follow-up (when the maximum decrease in CRP occurred) in the intervention group. During this time CRP decreased 1.43 mg/L, fasting glucose 0.47 mmol/L body weight 4.1 kg and fasting plasma insulin 11.2 pmol/L. Only the change in fasting glucose correlated to the change in CRP (p=0.031).
Figure 11
CRP levels during the study in the two groups.
CRP as a predictor of stroke. (Paper III)

Time from baseline screening to the event was, on average, 52 months for the 308 ischemic strokes and 50 months for the 61 intracerebral hemorrhagic strokes. 749 matched referents were randomly selected from the same population-based health surveys. In the intracerebral hemorrhage group 90% had hypertension and 72% were men.

Significant correlations were found between CRP and age, smoking, BMI, cholesterol, diabetes, and hypertension. The logistic regression analysis showed that CRP, hypertension, diabetes, cholesterol and BMI were significantly associated with ischemic stroke as outcome. In the multivariate model including smoking, hypertension, cholesterol, BMI, and diabetes, CRP (>3mg/L vs. <1mg/L) remained associated with ischemic stroke OR 2.06 (95% CI; 1.29 to 3.29). In stratified analysis this finding was consistent among men and women, hypertensives and normotensives.

In TOAST classified ischemic stroke, CRP (>3mg/L vs. <1mg/L) was significantly associated with large vessel disease, cardioembolic stroke, small vessel disease and undetermined stroke. In the multivariate model a significant association remained only for small vessel disease.

Hypertension was by far the strongest determinant for intracerebral hemorrhage with an OR of 12.7 (95% CI, 3.81 to 42.2). No significant associations between CRP and intracerebral hemorrhage were found.

The CRP 1444C>T genotype was significantly associated with plasma levels of CRP with highest levels observed in subjects with the CRP 1444TT genotype, median (interquartile range) 1.40 (0.95–2.96) vs. 1.09 (0.59–2.37) for the CRP 1444CC/CT group. In contrast, the CRP 1444 genotype (CC/CT vs. TT) was not associated with either ischemic stroke, intracerebral hemorrhage or total stroke.
PAI-1 mass as predictor for recurrence of atrial fibrillation (Paper IV)

In total, 129 patients with a mean age of 65 were eligible for the study. Table 2 shows that the studied subgroup was representative of the entire study population in baseline characteristics. There was no significant difference in studied variables.

Table 2
Baseline characteristics. Subgroup versus entire study are compared. Median and 25th and 75th percentiles are given.

<table>
<thead>
<tr>
<th>Number (n)</th>
<th>Subgroup</th>
<th>Entire study</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>129</td>
<td>67.0 (59.0; 73.5)</td>
<td>66.0 (59.0; 73.0)</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>97/32 (75.2)</td>
<td>176/58 (75.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Smokers n (%)</td>
<td>11 (8.5)</td>
<td>21 (9.0)</td>
<td>0.88</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.0 (76.5; 98.0)</td>
<td>88.0 (78.0; 98.0)</td>
<td>0.70</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.6 (25.1; 30.9)</td>
<td>27.8 (25.3; 30.9)</td>
<td>0.64</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.30 (4.80; 5.90)</td>
<td>5.20 (4.60; 5.90)</td>
<td>0.62</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.24 (1.05; 1.41)</td>
<td>1.24 (1.05; 1.43)</td>
<td>0.87</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.28 (2.78; 3.83)</td>
<td>3.18 (2.70; 3.74)</td>
<td>0.45</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.58 (1.02; 1.88)</td>
<td>1.45 (1.08; 1.87)</td>
<td>0.56</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>54 (41.9)</td>
<td>112 (47.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>Stroke n (%)</td>
<td>5 (3.9)</td>
<td>11 (4.7)</td>
<td>0.72</td>
</tr>
<tr>
<td>ICH n (%)</td>
<td>8 (6.2)</td>
<td>10 (4.3)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Effect of cardioversion and rhythm

Two days after cardioversion, PAI-1 activity, PAI-1 mass, and tPA mass had decreased significantly and CRP had increased significantly compared to baseline. Among the 53 individuals who remained in sinus rhythm after 30 days, PAI-1 activity, tPA activity, and tPA mass was significantly lower at day two after cardioversion compared to baseline and in the group with atrial fibrillation, PAI-1 mass was significantly lower. CRP was significantly lower.
at day 30 as compared to baseline among those who remained in sinus rhythm. There were no significant differences for any fibrinolytic variable, vWF or CRP, either at baseline or at day 30 between those who remained in sinus rhythm and those who had recurrent AF at follow-up.

**Predictors of rhythm**

In univariate logistic regression, no fibrinolytic variable, CRP level or vWF level was significantly correlated with rhythm at day 30. In the multivariate analysis (including PAI-1 mass, t-PA mass, t-PA activity, CRP, triglycerides, total cholesterol, hypertension, BMI, age and smoking), lower baseline PAI-1 mass was significantly associated with sinus rhythm at day 30. Lower baseline PAI-1 mass was also related to sinus rhythm at day 2 and remained significant in the multivariate model. After adding treatment allocation to the multivariate model, PAI-1 mass remained significantly associated with sinus rhythm both at day 2 and 30.
<table>
<thead>
<tr>
<th>Paper</th>
<th>Design</th>
<th>Population</th>
<th>Studied markers</th>
<th>Intervention</th>
<th>Endpoint/ Diagnosis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Andersson J, et al. Effects of heavy endurance physical exercise on inflammatory markers in non-athletes. <em>Atherosclerosis.</em> 2010 Apr; 209(2):601-5</td>
<td>Observational intervention cohort study.</td>
<td>Twenty fit, non-athletic men aged 18-55 years.</td>
<td>CRP, IL-6 and TNF-α levels analysed at baseline, during physical exercise and 4, 6 weeks after intervention.</td>
<td>Physical intervention through a 14 day long cross country skiing tour.</td>
<td>Change in CRP, IL-6 and TNF-α levels at six weeks after end of exercise.</td>
<td>CRP and TNF-α increased significantly but reacted differently during heavy physical activity. No change was observed for IL-6.</td>
</tr>
<tr>
<td>(II) Andersson J, et al. Effect of intensive lifestyle intervention on CRP in subjects with impaired glucose tolerance and obesity. <em>Biomarkers.</em> 2008 Nov;13(7):671-9.</td>
<td>Randomized controlled trial</td>
<td>193 patients with BMI &gt;27 kg/m² and impaired glucose tolerance recruited from the Västerbotten intervention programme.</td>
<td>CRP levels at baseline, one, three and five years.</td>
<td>Lifestyle intervention through a 1-month stay at a wellness centre.</td>
<td>Change in CRP. Participants followed for five years.</td>
<td>No change was observed between the groups. CRP was significantly lower after 1 (p=0.001) but not 3 years within the intervention group.</td>
</tr>
<tr>
<td>(III) Andersson J, et al. C-Reactive Protein Is a Determinant of First-Ever Stroke. <em>Cerebrovasc Dis.</em> 2009Apr; 27(6):544-551</td>
<td>Prospective nested case-referent study.</td>
<td>308 ischemic stroke, 61 intracerebral haemorrhages and 749 referents. Mean age 55 years at inclusion.</td>
<td>CRP levels at health examination.</td>
<td>None.</td>
<td>OR for CRP &gt;3mg/L versus &lt;1 mg/L was 2.58 (95% CI 1.74–3.84) for ischemic stroke and 1.63 (95% CI 0.67–3.93) for hemorrhagic stroke.</td>
<td></td>
</tr>
<tr>
<td>(IV) Andersson J, et al. Markers of fibrinolysis and vWF as predictors for recurrence of atrial fibrillation after electrical cardioversion. <em>Thrombosis Research.</em> 2010</td>
<td>Pre-specified hypothesis generating study within a randomized controlled trial.</td>
<td>129 patients with atrial fibrillation, mean age 65 years.</td>
<td>PAI-1 activity, PAI-1 mass, tPA activity, tPA mass and CRP at baseline and at 2 and 30 days after cardioversion.</td>
<td>Substudy of a randomized study, 80 mg Atorvastatin vs. placebo.</td>
<td>Sinus rhythm 30 days after cardioversion.</td>
<td>PAI-1 mass was significantly associated with sinus rhythm both at day 2 and 30 (OR 0.98; 95% CI 0.95-1.00) in the fully adjusted model.</td>
</tr>
</tbody>
</table>

Table 3
Compilation of papers I-IV.
DISCUSSION

Populations

Paper I

With the objective of exploring the effects of endurance physical activity on inflammatory markers we chose a population of healthy men. The group leader was an experienced tour guide and general practitioner and selected those participants he knew could manage the tour. The external validity of the study is low due to the selection process, but the purpose was to study mechanisms. All participants completed the study, at least partly, due to the selection process. The focus on analysis of mechanisms also meant that the number of participants could be kept quite low and still provide useful information. The comparatively low number of participants was mainly due to the logistics of performing such a physically demanding skiing tour (including sampling and dietary preparation) and also for safety reasons. Women were not included in the study which means that the results only reflect the male gender. An advantage is that the possible effects of the hormone cycle, menopause and hormone replacement therapy did not have to be regarded. Initially a study on women was also intended but it was never conducted due to economical and administrative circumstances. Further studies of dietary effects and gender on inflammation during physical exercise are needed.

Paper II

Inflammatory markers are known to be increased among obese individuals with impaired glucose tolerance. The chosen population of individuals with obesity and impaired glucose tolerance are of clinical interest as large health benefits can be achieved if CVD risk is reduced. The participants were recruited from the VIP in which men and women upon reaching 30, 40, 50 and 60 years of age in Västerbotten County are invited to participate in a screening program for CVD and diabetes. The population is representative of northern Sweden. A limitation is that only 345 of the 650 who fulfilled the inclusion criteria were interested in participating. It is a challenge to put one’s life on hold for an entire month. This may have discouraged potential participants. This may constitute a potential selection bias.
Paper III

Study III is based on the Northern Sweden cohorts consisting of the VIP and the Northern Sweden MONICA study cohorts. The screening centres for the VIP, which provided the majority of the screened subjects, are distributed around the county of Västerbotten. Selection bias for social and health factors in the VIP was assessed for participants in 1992 and 1993. Differences in social characteristics between participants and non-participants indicated that the social selection bias was small. A somewhat lower preference to participate was found for those with low income, younger age or joblessness. In general, the health factors were found to be quite similar, but participants in the VIP had lower mean total cholesterol and their blood pressure was generally higher. The VIP only included subjects aged 30-60 years and MONICA 25-74 years, limiting the external validity, especially for older populations where stroke is more common. Cases with myocardial infarction or cancer were excluded as they were included in other studies.

Paper IV

With the primary aim of exploring the role of fibrinolytic factors as predictors of maintenance of sinus rhythm after electrical cardioversion with adjustment for CRP and metabolic factors, patients were recruited in a multicentre study. Five participating centres collected blood samples from 129 patients with a duration of atrial fibrillation more than 7 days between August 2004 and January 2007. Patients under 18 years and over 80 years of age were excluded, as were patients with paroxysmal AF, treatment with lipid-lowering drugs or ongoing treatment with class I or class III antiarrhythmic drugs. Lipid-lowering drug use as an exclusion criterion probably contributed to a population representing a lower cardiovascular risk. A rather large proportion of screened patients, 39%, were also excluded of this reason in the main study. Further research is needed to clarify if our results are applicable to those patients with atrial fibrillation taking statins.
Study designs

**Paper I**

In study one an observational cohort study design was used. As the study objective was to observe changes in inflammatory variables during and after heavy endurance physical exercise it was important to control the exercise performed. The subjects were on the same tour so compliance to exercise is indisputable. The level of difficulty was mainly the same for every participant independent of previous physical fitness. The selection of study subjects required that they be fit and able to perform the tour which was designed to be strenuous even for trained individuals. Furthermore, diet was rigidly controlled as each participant had his diet packed in advance randomized to 30 or 40 energy percent fat by a dietician, but there were no limitation to total energy intake for each individual.

One limitation is that blood samples were only performed five times during the study. Closer monitoring, especially during the first hours and days of exercise, would have been of interest as many inflammatory markers respond within hours. This was not practically feasible due to the conditions during which the study was preformed. The dietary groups were small which markedly hamper conclusions on the effects of diet fat composition during exercise.

The analytic sensitivity constituted another limitation as IL-6 levels are very low in healthy subjects; the numbers of subjects with IL-6 levels below the detection limit of 2 pg/ml were 15 at baseline, 11 at week one, 9 at week two, 16 at week six and 15 at week eight of the 20 participants. Early changes in IL-6 were not observed due to the limited number of blood samples during the study as half-life of IL-6 is very short. The results should reflect the effects of heavy endurance physical exercise on CRP, IL-6 and TNFα in healthy, non-athletic, male subjects.

**Paper II**

Study II was performed as a randomized controlled trial with change in CRP levels as outcome. The intensive intervention programme had an extensive, all-embracing focus on healthy lifestyle including daily low to moderate intensity exercise, a high fibre, low-fat content diet, health promoting coping strategies, stress management and relapse prevention techniques. This approach does not allow determination of individual components in the programme on CRP levels, but instead mirrors the effects of the combined
lifestyle change on CRP levels in this population. Neither the mechanisms involved nor the potential health benefit related to reduced CVD risk can be answered by this study design. To study if the effect on CRP levels translates into a reduced risk for CVD requires a considerably larger sample size and would have necessitated a far greater expense. The control group underwent a counselling session for 30-60 minutes conducted by a specially trained nurse and were given both oral and written advice focusing on lifestyle intervention, but they had to find time independently to introduce exercise and dietary modifications. The differences between the groups are primarily the time to start up a healthy lifestyle and available support during that time. An alternative control group could have had weekly meetings during one month so that problems and questions arising could have been answered and information repeated. A limitation to the study was that the intervention group had a higher BMI, CRP levels and lower triglycerides and fasting plasma insulin levels than the control group at baseline. This has to be considered during interpretation of the results. We have found no specific cause and assumed this was a random phenomenon.

**Paper III**

To study CRP as a determinant for stroke a prospective population-based, nested, case-referent study design was used. The prospective design means that blood samples had been drawn before the stroke event which reduces the risk for reverse causation, i.e. that stroke caused elevated CRP levels. The prospective design also allows fatal cases to be included, in contrast to a retrospective, case-control design. The nested case-referent design is more cost-effective than a normal cohort study as only cases and matched controls have to be analysed. The study does not consider current lipid lowering medication which may have affected CRP levels. However, as the population had no previous myocardial infarction or stroke the number of individuals on such treatment were probably limited. The questionnaire did not include other health conditions that may induce inflammatory responses such as infections. The single measurement of the analyzed CRP adds to this limitation. The intracerebral hemorrhage group and TOAST classified subgroups are small due to a strict subtyping process which reduces statistical power. Type two errors cannot be ruled out.

**Paper IV**

Study IV was performed as a pre-specified hypothesis generating study within a randomized, controlled, multicentre study. As an explorative substudy, the results should be regarded as hypothesis generating. The number of patients is a limitation; the study is underpowered to detect
weaker associations. Another limitation is that asymptomatic paroxysmal atrial fibrillation was not detected unless it coincided with the follow-up. Patients were randomized to 80 mg atorvastatin or placebo. This randomization was not necessary for the present study which could have been performed as cohort study instead of the randomized controlled study design. To adjust for the treatment randomization, the results have be adjusted for treatment (atorvastatin or placebo) in the multivariate analysis.
Main results

**Inflammation and physical activity in cardiovascular medicine**

Physical activity is recognised to reduce the risk for CVD, but the mechanism is not fully understood. In paper I, we found that physical activity reduced CRP in healthy subjects. A study by Mora showed that the inverse association between physical activity and CVD risk was explained primarily by inflammatory and haemostatic factors (32.6%) followed by blood pressure (27.1%). It is possible that anti-inflammatory effects, at least in part, explain how physical activity is associated with the risk of CVD.

The effect of physical activity on inflammation is complex. A sudden increased physical activity may result in overtraining and exhaustion causing an inflammatory reaction. The inflammatory response from intense exercise also seems to depend on the type of exercise. Such an inflammatory response may explain the initial increase in CRP and TNF-α during the first week of training in paper I. During the second week of training, CRP started to decrease which may be caused by adaptive and anti-inflammatory responses. It is interesting to note that six weeks after the exercise, CRP levels remained lower than baseline. A longer follow up would have been interesting. The anti-inflammatory effect of physical activity may have both a delay to onset and a prolonged effect after the training has ceased. It is also possible that the study in some way changed the participant’s lifestyle, thereby contributing to the lower CRP levels even after six weeks.

TNF-α, in comparison to CRP, continued to increase throughout the exercise period and then returned to baseline. TNF-α can induce CRP production but, does not explain CRP changes in this study. IL-6 is well known to induce CRP production but did not change significantly during the exercise in our study. The only significant change in IL-6 was from the second week of exercise, with the highest IL-6 levels, to the end of the study. IL-6 levels are lower in more physically active subjects which may explain the low IL-6 levels seen in our study. As neither TNF-α nor IL-6 alone can explain changes in CRP levels it is reasonable to assume that other adaptive or anti-inflammatory cytokines as IL-10 are involved. We must also recognise the inflammatory modulating properties of IL-6. It may not only act pro-inflammatory, but also anti-inflammatory. IL-6 can be derived from muscles, as well as adipose tissue. If the effect of IL-6 production in muscles during physical exercise is different from that in adipose tissue among obese individuals remains to be answered. We hypothesise that there is a slow adaptive inflammatory equilibrium that can be altered depending on the
level of physical activity. The change in this inflammatory equilibrium through physical exercise may result in decreased levels of inflammatory markers, which in turn may explain the reduced risk for CVD through physical exercise.

The reduction in CRP in paper I seemed independent of changes in weight and metabolic variables. And in paper II, CRP reduction was only associated to the reduction in fasting glucose levels. Physical activity thus seems to be able to decrease inflammation independent of changes in weight and metabolic variables which is supported by previous studies.\textsuperscript{82, 84, 89} This finding is, however, limited to the studied populations and non-linear associations may also exist.

In paper I, CRP reduction was achieved through physical exercise in a healthy group with normal CRP levels. Physical exercise is also beneficial for risk group subjects. In paper II we found that CRP levels could be reduced through a combination of lifestyle changes, including physical activity, in an obese population with impaired glucose tolerance. The beneficial effect of physical activity on individuals with CVD is well recognized for reducing cardiac mortality.\textsuperscript{138-140}

**Inflammation, obesity and type 2 diabetes in cardiovascular medicine**

Systemic immune mediators are associated with progression to type 2 diabetes in obese subjects with impaired glucose tolerance.\textsuperscript{141} The finding in paper II, namely reduced CRP levels through comprehensive lifestyle change including diet, exercise, stress management, and smoking cessation imply a relationship between metabolism and inflammation in obese subjects with impaired glucose tolerance. The participants in paper II also reduced the progression to type 2 diabetes after one, but not three or five years.\textsuperscript{142} The study baseline CRP levels correlated to body mass index.

Paper II does not address the causes of reduced CRP levels, but there are several possibilities. Weight loss can reduce CRP levels independent of type of intervention, lifestyle and surgical intervention alike.\textsuperscript{93} Exercise can also reduce CRP levels independent of weight loss in type 2 diabetics with the metabolic syndrome.\textsuperscript{143} Smoking cessation\textsuperscript{144} and improved alcohol habits\textsuperscript{145} may also contribute to lower CRP levels. Paper II supports lifestyle change as a non-pharmacological measure to reduce elevated CRP levels among obese with impaired glucose tolerance, a group with increased risk for CVD.
In paper I, CRP reduction from baseline to the end of the study was only observed among those randomized to 40 energy percent from fat in the diet. This observation warrants further studies of the dietary effects on the inflammatory response during physical exercise.

**CRP and stroke**

Stroke is a highly heterogeneous disorder. In paper III we found that elevated CRP levels was a predictor of ischemic stroke, a finding supported by previous studies. However, when studying subgroups of ischemic stroke, CRP was most strongly related to small-vessel disease. Stroke subtypes have not been regarded in previous studies, but there are some support for the finding of a stronger relation between CRP and small-vessel disease in the PROGRESS study and the Rotterdam Scan study. The subgroups were small in our study and a correlation to other subgroups can not be ruled out due to the possibility of type 2 errors.

In paper III we found no relation between CRP and intracerebral hemorrhage. We have identified two previous studies on this subject both supporting our finding. Considering that intracerebral hemorrhage and ischemic stroke have different risk factors and disease mechanisms this finding is reasonable.

The CRP1444TT genotype studied in paper III was associated with increased levels of CRP, but not to stroke. This finding, a form of Mendelian randomization, indicates that the relation between CRP and stroke is not causal and this is supported by previous studies.

**Inflammation, haemostasis and atrial fibrillation**

The pathophysiology behind atrial fibrillation is not fully understood. Since the 1990s there has been an increasing interest in the inflammatory mechanisms possibly involved.

In paper IV no fibrinolytic component or CRP was found to be a predictor of recurrence of atrial fibrillation in univariate analysis. In multivariate models, lower PAI-1 mass was associated with sinus rhythm even after adjusting for CRP and markers of the metabolic syndrome, suggesting a link between atrial fibrillation and PAI-1 mass, a finding which is coherent with previous studies. Atrial fibrillation causes disturbances in the fibrinolytic system possibly contributing to the prothrombotic state observed in this arrhythmia. The changes seem to be rapid as fibrinolysis improved within two days after electrical cardioversion in paper IV. Inflammation,
endothelial dysfunction, hypertension and insulin resistance have all been proposed to influence fibrinolytic function in atrial fibrillation.\textsuperscript{47, 52}
Clinical utility

**In prevention**

Physical activity and the maintenance of a normal body weight throughout life are key factors in avoiding the development of metabolic disorders and cardiovascular diseases. The findings in our studies further support the importance of physical exercise. We also show that among obese subjects with impaired glucose tolerance, which have both increased CRP levels and increased risk for CVD, comprehensive lifestyle change can be used to significantly lower CRP levels. Our results need confirmation and further research is needed to see if the effect on CRP levels translates to decreased risk of developing cardiovascular disease.

**In clinical practise**

The JUPITER trial in 2008 brought CRP closer to clinical utility as a guide for initiation of statin treatment to limit cardiovascular events. Inflammation thereby moved towards practical clinical utility in risk assessment and CRP emerged as a potential target for therapy. However, the study only address the preventive effects of statin drugs with regard to CVD among healthy subjects with normal LDL levels and increased levels of CRP, leaving the question of causality unanswered. The study may actually only imply that current LDL goals are not low enough.

The American Heart Association had guidelines for CRP assessment in cardiovascular risk prediction even before the Jupiter study. CRP was found to have its greatest value in primary prevention among an intermediate risk group. Reynolds Risk Score is another risk scoring tool for calculating the CVD risk and is available without cost at http://www.reynoldsriskscore.org. Experiences from Linköping University Hospital, where CRP was routinely followed during follow-up visits after acute coronary syndromes, show that statin treatments and other interventions made interpretation difficult and clinical value was limited. The AHA statement are in line with these experiences stating that application of management guidelines for acute coronary syndromes and secondary prevention measures should not be dependent on CRP levels. Swedish cardiologists have a generally reserved approach to utilizing CRP for CVD prediction. This position is supported by a recent review that does not support the routine use of CRP for risk stratification of intermediate risk persons.
**In risk stratification**

Our studies also show that CRP constitutes a possible risk marker for all subtypes of cerebral infarction except those of rare causes (e.g. haematological disorders) but the strength of the relationship is different among subtypes of stroke. These results add to previous knowledge on CRP as a risk marker for CVD.

C-reactive protein can thus be used in the risk stratification of cerebral infarction, but similar to CVD where CRP has been found to be a risk marker, causality is unclear or suggestive of a non-causative relationship. Inflammatory markers still have, regardless of causality, a value in improving risk stratification. This is important because we observe serious CVD affecting patients despite favorable risk calculations such as the Framingham Risk Score. CVD remains the leading cause of death in Sweden. Further research is needed to learn which preventive measures are suitable for an individual with increased risk according to CRP, but a healthy lifestyle would be a good start.
Future perspectives on inflammation and cardiovascular disease

CRP as a causal factor in the pathogenesis of atherosclerosis is among the most discussed subjects in cardiovascular medicine today. Results from Mendelian randomisation studies\textsuperscript{74, 75} contradict a causal association, but when the Jupiter study\textsuperscript{165} demonstrated that statin drugs can reduce CVD among subjects with normal cholesterol but increased CRP levels the debate was revived.

A randomised controlled trial with a CRP antagonist and mortality as outcome would give further insight in the casual nature of the relationship. For this purpose several methods of reducing CRP have been suggested. These include transcriptional inhibition of hepatic CRP synthesis, anti-sense strategies and blockage of CRP-mediated complement activation or CRP receptors\textsuperscript{167}. Regardless of the causal role of CRP in atherosclerosis increasing knowledge in the pathophysiology behind atherosclerosis can improve risk scores, prognostication, and ultimately lead to new treatment regimes.

The inflammatory processes in atherosclerosis are complex involving a myriad of proteins. CRP might have gained too much focus. In many analytic aspects it is a suitable inflammatory marker, but it does not bring us closer to understanding the interactions between inflammation and atherosclerosis. The moderate elevations of CRP presently in focus also seem inappropriate to be characterised as a systemic inflammation as the levels are within a physiologic range and the study subjects lack symptoms of inflammation.

Increased knowledge of the effects of physical exercise on inflammatory markers is important as the preventive effects from physical activity on CVD could be mediated through inflammation. We need to understand how intensity and duration of physical exercise affects the inflammatory response and if the reduction of inflammatory markers carries additional information beyond known effects on weight, cholesterol levels, insulin resistance and blood pressure. It is therefore interesting that Paper I and II fail to explain the change in CRP levels in relation to weight and cholesterol. Future studies will require more awareness of the potential influence of diet and gender on the inflammatory response during endurance physical exercise, as current knowledge is limited.

C-reactive protein is fairly established as a cardiovascular risk marker. This is supported by several large, prospektive and well-controlled trials.
Improved knowledge is, however, still needed on CRP as a risk marker for different subtypes of ischemic stroke and for specific CVD risk groups such as obese and type 2 diabetics. The organisation of large multinational cohorts presently assembled for Mendelian randomisation studies on CRP genotypes constitute a suitable basis for addressing such questions.

Treatments to decrease levels of inflammatory markers may, if successful, constitute a novel way to increase the success rate and maintenance of sinus rhythm during rhythm control management of atrial fibrillation. PAI-1, a component of the fibrinolytic system with acute phase response properties, is associated with maintenance of sinus rhythm following electrical cardioversion. It may become a means to assist the choice between the many treatments for atrial fibrillation presently available, such as avoiding adverse events from treatment options with low success rate for a particular individual. Further understanding of the relationship is needed and future investigation of atrial fibrillation treatment guided by PAI-1 or inflammatory markers would be of special interest.
CONCLUSIONS

CRP and TNF-α increased significantly during heavy endurance physical exercise but CRP peaked already during the exercise. In the recovery phase at the eighth week CRP, but not TNF-α, was significantly lower than baseline. For IL-6 there was no significant change during the study. No significant differences regarding inflammatory variables were found between the dietary groups.

CRP can be decreased up to one year after intensive lifestyle intervention programme on subjects with obesity and impaired glucose tolerance.

CRP levels >3 mg/L compared to <1 mg/L are associated with a doubled risk of having a first ischemic stroke when controlling for traditional risk factors, a finding confirmed for the small-vessel disease subgroup.

No significant association was found between intracerebral hemorrhage and CRP.

There is a relation between the CRP 1444C> T single nucleotide polymorphism and plasma levels of CRP but not between the CRP 1444C> T single nucleotide polymorphism and stroke.

In univariate analysis no component of the fibrinolytic system was found to be a predictor for maintenance of sinus rhythm. In multivariate models with fibrinolytic variables lower PAI-1 mass was associated with maintenance of sinus rhythm even after adjusting for CRP and markers of the metabolic syndrome.
ACKNOWLEDGEMENTS

"At times our own light goes out and is rekindled by a spark from another person. Each of us has cause to think with deep gratitude of those who have lighted the flame within us."

- Albert Schweitzer, honoured for his humanitarian work with the Nobel Peace Prize 1952

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