Studies on inflammation and atherosclerosis in the metabolic syndrome

Marius Trøseid
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Series of dissertations submitted to the Faculty of Medicine, University of Oslo No. 920


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Cover: Inger Sandved Anfinsen.
Printed in Norway: AiT e-dit AS.

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I. Acknowledgements

The incitement of this thesis was my attendance at two international congresses in 2001, first the 50th session of American College of Cardiology in Orlando, where pravastatin was reported to have an anti-inflammatory effect, and later the same year, the 72nd Congress of the European Atherosclerosis Society congress in Glasgow, where visceral adipose tissue was introduced as a potential source of inflammation in subjects with the metabolic syndrome. Russel Ross had just introduced the concept of atherosclerosis as an inflammatory disease two years earlier, and several questions were now raised: Is the metabolic syndrome an inflammatory state, and what does this mean for the development of atherosclerosis? Does adipose tissue play a part in this process? If statins can modulate inflammation, what about life-style modifications that may reduce the amount of adipose tissue? The hypotheses of the present thesis were gradually being formulated.

This work was carried out during two periods, first from 2001 to 2004 at Nordland Hospital, Bodø, and then from 2007 to 2009 at Ullevål University Hospital, Oslo, also in collaboration with the milieu in Bodø. In the first period, I was supported by H. Hofstad Berg’s memorial foundation, and in the second period, I received a research fellowship from Helse Sør-Øst (South-Eastern Norway Health Authority) and financial support from Stein Erik Hagen’s Foundation for Clinical Heart Research, for which I am very grateful.

First of all, I want to express my sincere gratitude to my supervisors Professor dr. med. Harald Arnesen and Professor dr. philos. Ingebjørg Seljeflot, both showing an extraordinary combination of skills, patience, wisdom and enthusiasm. They have always been available, either for ventilation of new ideas, practical support, valuable comments or teaching. I also want to express my sincere gratitude to Professor Knut Tore Lappegård and Professor Tom
Eirik Mollnes, both for backing my initial curiosity and for helping me through all the
difficult steps of transforming ideas into a research project and first international publication.

A large number of people have been involved in the two studies that form the basis of my
thesis, and without their support this work would not have been possible. First, I am most
grateful to Dr. Elsa M. Hjerkinn, for having collected data for the excellent database in the
DOIT study. I am in debt to the skilled laboratory staff in Bodø and Ullevål Hospitals,
especially medical technologist Sissel Åkra for performing the biochemical analyses from the
large DOIT database, and to Kari Lise Aakre, Grethe Bergseth, Dorte Christiansen, Gry
Edvardsen and Hilde Fure for skilful technical assistance in my first project. Also special
thanks to clinical nutritionist Ingrid Ellingsen for the dietary intervention in the DOIT study,
and to the staff at S.A.T.S. in Bodø for helping me with the exercise intervention. I want to
thank Leiv Sandvik for statistical support and interesting conversations, and all my co-authors
and co-workers in Bodø and Oslo for valuable and enthusiastic collaboration. Also thanks to
Malene Gundersen at the Ullevål library for valuable help with literature search and layout of
several figures.

I am also indebted to the research fellows and office neighbours Åse Sagen, Camilla Lund
Sørås, Anne Cecilie Larstorp, Inger Ariansen, Eva Cecilie Knutsen, Trine Andersen and Alf
Aage Pettersen for nice lunch breaks and solution of day-to-day challenges. Special thanks to
my good friend, co-author and colleague Thomas Weiss, for valuable help, interesting
discussions and a lot of fun.

Finally, I want to thank my friends and family for their support and patience, especially
my children Tuva and Rasmus who have put my research and professional life into
appropriate perspective, and to my beloved wife Åsa for being my best friend.
2. **Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ATP III</td>
<td>Adult Treatment Panel III</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CT</td>
<td>computerised tomography</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
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<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
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<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
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<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
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<tr>
<td>IFG</td>
<td>impaired fasting glucose</td>
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<td>IGT</td>
<td>impaired glucose tolerance</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
</tr>
<tr>
<td>MCP-1</td>
<td>monocyte chemoattractant protein-1</td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td>omega-3 polyunsaturated fatty acid</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor type-1</td>
</tr>
<tr>
<td>PPAR</td>
<td>peroxysome proliferator activated receptor</td>
</tr>
<tr>
<td>PWP</td>
<td>pulse wave propagation</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-α</td>
</tr>
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<td>WHO</td>
<td>World Health Organisation</td>
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3. List of papers


V. Trøseid M, Seljeflot I, Weiss TW, Klemsdal TO, Hjerkinn EM, Arnesen H. Arterial stiffness is independently associated with interleukin-18 and components of the metabolic syndrome. *Atherosclerosis* 2009; Sep 25. E-pub.
4. Introduction
4.1. Classification and epidemiology
4.1.1. The metabolic syndrome

The metabolic syndrome is a cluster of risk factors for cardiovascular disease (CVD), including abdominal obesity, elevated glucose, hypertension, elevated triglycerides and low levels of high-density lipoprotein (HDL) cholesterol. The syndrome has received increased attention after practical and updated definitions by the Adult Treatment Panel III (ATP III) and the International Diabetes Federation (IDF) (Table 1) (1-3). Although other classifications exist, and the criteria vary to some degree, all definitions identify a population with increased risk for developing type 2 diabetes mellitus and CVD (4-6).

Table 1. Common definitions of the metabolic syndrome. Adapted from Grundy SM (7).

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Insulin resistance or diabetes/impaired glucose tolerance (IGT)/impaired fasting glucose (IFG)* plus 2 of:</td>
<td>At least 3 of the following:</td>
<td>Waist circumference ≥ 94 (men)/ 80 (women) cm*** plus 2 of:</td>
</tr>
<tr>
<td>Blood pressure ≥ 140/90 or treatment.</td>
<td>• Waist circumference≥102 (men)/88 cm (women)</td>
<td>• Blood pressure ≥ 130/85 mmHg or treatment.</td>
</tr>
<tr>
<td>Triglycerides ≥ 1.7 mmol/L or HDL &lt; 0.9 (women)/1.0 (men) mmol/L.</td>
<td>• Triglycerides ≥ 1.7 mmol/L (women) mmol/L</td>
<td>• Triglycerides ≥ 1.7 mmol/L or treatment.</td>
</tr>
<tr>
<td>BMI ≥ 30 kg/m2 or waist/hip ratio &gt; 0.9 (men)/0.85 (women)</td>
<td>• Blood pressure ≥ 130/85 mmHg or treatment.</td>
<td>• HDL &lt; 1.0 (men)/1.3 (women) mmol/L.</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>• Glucose ≥ 5.6** mmol/L or treatment.</td>
<td>• Glucose ≥ 5.6 mmol/L or treatment.</td>
</tr>
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</table>

*See table 2 for definitions of IGT and IFG. ** Cut-off changed from 6.1 mmol/l (2001) according to updated definition of IFG. ***The IDF definition has population specific cut-offs for waist circumference.
Whereas the original definition by Reaven (1988) (8), as well as the definition by the World Health Organisation (1998) emphasised insulin resistance as mandatory for the diagnosis, no measure of insulin resistance is present in the updated definitions by IDF and ATP-III (2005) (9). Instead, the role of central obesity measured by waist circumference has been given more attention, and is mandatory by the IDF criteria. A few comparative studies have aimed to compare the various definitions, and it seems that the IDF-definition identifies slightly more individuals with the syndrome (10-12).

Physical inactivity and increased caloric intake have led to an emerging epidemic of obesity. In the United States abdominal obesity has tripled during the past 40 years (13), more than 25% of the US population can be classified as having the metabolic syndrome (14), and the prevalence is increasing (15). Depending on which classification that has been used, similar prevalence of the syndrome can be found in India and several countries in Europe, whereas the prevalence is even higher in some Latin-American countries (21 to 43%) and lower in South-East Asia (7). The prevalence is also increasing with age, affecting >40% of US adults above the age of 60 years (16). Similar age-related prevalence has been shown in a Norwegian cohort, increasing from 13% in the 20-29 year age group to 41% in the 70-79 years age group in men, and from 6% to 51% for women in the corresponding age groups (ATP III criteria) (Figure 1) (17).

As all the individual components of the syndrome have been shown to increase the risk of CVD, including elevated fasting glucose (18), abdominal obesity (19), hypertension (20), elevated triglycerides (21) and low levels of HDL cholesterol (22), it has been discussed if the metabolic syndrome is a useful clustering of risk factors (23), and if the
syndrome really exists (24). Furthermore, different combinations of these components might identify very different phenotypes, although the diagnostic criteria of the syndrome are fulfilled (9).

Figure 1. Age-specific prevalence of the metabolic syndrome in Norway. Adapted from Hildrum B (17).
Table 2. American Diabetes Association criteria for diagnosis of diabetes and impaired glucose regulation. Adapted from Genuth S (25).

<table>
<thead>
<tr>
<th>Category</th>
<th>Fasting plasma glucose</th>
<th>2-hour post-load plasma glucose</th>
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<tbody>
<tr>
<td>Normal</td>
<td>&lt; 5.6 mmol/L</td>
<td>&lt; 7.8 mmol/L</td>
</tr>
<tr>
<td>Impaired fasting glucose (IFG)</td>
<td>5.6-6.9 mmol/L</td>
<td>-</td>
</tr>
<tr>
<td>Impaired glucose tolerance (IGT)</td>
<td>-</td>
<td>7.8-11.0 mol/L</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>≥ 7.0 mmol/L</td>
<td>≥ 11.1 mmol/L</td>
</tr>
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</table>

As an example, elevated fasting glucose is a more useful marker for increased risk of diabetes mellitus than any of the other components (9, 26). The metabolic syndrome is a strong predictor of type 2 diabetes, with an increased incidence rate of 5 to 7-fold (26, 27). Indeed, the increased cardiovascular risk might develop as a continuum in parallel with increasing fasting glucose, from the normal range via impaired fasting glucose to overt diabetes mellitus (Table 2) (28).

The risk of developing CVD is approximately doubled in the metabolic syndrome (7). Importantly, subjects with the syndrome may be classified as having low risk of CVD by both the Framingham score and the European Systematic Coronary Risk Evaluation (SCORE) but still be at increased risk of subclinical atherosclerosis and cardiovascular events (29-31). In a recent meta-analysis including 43 cohorts, the relative risk for cardiovascular events and death was 1.78, with the highest risk in women (relative risk 2.63) (32). After adjustment for traditional risk factors like hypercholesterolemia and smoking, the syndrome was still associated with increased risk (relative risk 1.54).
4.1.2. Atherosclerosis

Atherosclerosis is a systemic disease affecting large and medium-sized arteries, with dominating clinical symptoms from the heart, brain and extremities (33), including the aorta. Atherosclerotic lesions are asymmetrical focal thickenings of the innermost layer of the intima, consisting of cells, connective-tissue elements, lipids and debris (34). The lesions tend to develop in areas in the vasculature with disturbed non-laminar flow (35).

Most cardiovascular events occur with the formation of a thrombus on the plaque surface (36). Thrombosis is caused by either endothelial erosion or rupture of the plaque. Plaque rupture occurs most often in thin and partly destroyed parts of the fibrous cap, exposing pro-thrombotic material such as tissue factor and platelet-adhesive matrix molecules to the circulation (37).

Atherothrombosis underlies by far most cases of coronary heart disease and peripheral arterial occlusive disease, as well as many cases of stroke (38). Thus, atherosclerosis and its thrombotic complications are the main causes of death and disabling diseases in Europe, the United States and much of Asia (39, 40). At present, CVD cause almost 40 percent of all deaths in North America and are the most common cause of death in European men under 65 years, and the second most common cause in women (41). Furthermore, due to a rapidly increasing prevalence of obesity and diabetes, CVD is expected within the coming years to be the leading cause of death globally (39, 40).

The progression of atherosclerosis is partly dependent on genetic susceptibility and the presence of risk factors such as smoking, hypertension, diabetes mellitus and dyslipidemia
(38). Elevated plasma levels of low density lipoprotein (LDL) cholesterol have been one of the principal cardiovascular risk factors, and the atherosclerotic lesion has previously been thought to largely consist of lipid accumulation within the arterial wall (33). Notably, half of coronary events occur in persons with normal lipid levels (39), and our understanding of the atherosclerotic process has changed substantially during the last two decades.

4.2. Inflammation in atherosclerosis and the metabolic syndrome

4.2.1. Atherosclerosis - an inflammatory process

It is now evident that inflammation plays an essential role in atherosclerosis, and leukocyte infiltration into the vascular wall is involved in virtually all stages of the process, from the fatty streak to the complex rupture-prone plaque (33). The atherosclerotic process starts in early life, with T-lymphocytes and lipid-containing macrophages (foam cells) accumulating beneath the endothelium, gradually being organised in layers to form fatty streaks, which are asymptomatic lesions that may progress to atheromata or disappear (41-43). In the stable atherosclerotic lesion, inflammatory cells are present in the shoulder region of the atherosclerotic plaque, in which the atheroma grows, whereas in the unstable plaque, activated immune cells preferentially occur in thin, rupture-prone areas (41).

The endothelium does normally not support binding and transmigration of leukocytes, but might be dysfunctional and leaky by exposure to several injuries such as hypertension, hyperglycemia, free radicals from smoking and modified lipids (33, 44). Modified lipids, such as oxidised LDL have been shown to induce expression of cellular adhesion molecules,
Figure 2. Oxidation of LDL cholesterol and activation of endothelium and macrophages. 
Spikes on endothelium surface represent adhesion molecules. Adapted from Hansson G (41).

such as E-selectin, P-selectin, intercellular adhesion molecule-1 and vascular cellular 
adhesion molecule-1, on the surface of endothelial cells (Figure 2) (45-48). Leukocytes and 
platelets from the blood stream attach to these adhesion molecules, and once attached, 
chemokines such as interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) 
stimulate the transmigration of monocytes and T-lymphocytes to the subendothelial space 
(49-51) (Figure 3). Inflammatory mediators such as MCP-1 and macrophage colony-
stimulating factor contribute to the differentiation of blood monocytes to macrophages, and to 
the expression of scavenger receptors leading to uptake of modified lipids and the formation 
of foam cells (52-54).
Figure 3. Transmigration of leukocytes. Spikes on endothelium surface represent adhesion molecules. Chemokines such as IL-8 and MCP-1 stimulate the adhesion and transmigration of leukocytes to the subendothelial space. Adapted from Hansson G (41).

In the atherosclerotic process, endothelial cells, smooth muscle cells, T-cells and macrophages have the potential to secrete proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-6, as well as IL-10 (33, 55, 56). T-lymphocytes are present in the atherosclerotic lesion, and when activated, a type 1 helper (Th1) response dominates, with production of a characteristic subset of inflammatory mediators, such as interferon-γ, IL-12, IL-18, TNF-α and CD40 ligand, which act in synergy to stimulate a cascade of cytokines from macrophages and vascular cells (41, 57).
Figure 4. The atherosclerotic plaque and adipose tissue as sources of systemic inflammation. Adapted from Hansson G (41).

Activation of the Th2 pathway leads to production of IL-4, IL-5 and IL-13, a cytokine subset which might have both pro- and anti-atherogenic effects (57-59). In addition, regulatory T-cells might produce anti-inflammatory agents such as IL-10 and transforming growth factor β which might dampen the inflammatory process (57, 60-63).

The inflammatory process in the atherosclerotic plaque may increase blood levels of inflammatory cytokines downstream in the cytokine cascade (Figure 4). In particular, C-reactive protein, which is produced in the liver in response to inflammatory stimuli like IL-6,
has been consistently shown to be elevated in patients with coronary events (64, 65), and there is evidence for a direct role in the atherosclerotic process (66, 67).

IL-18 is a potent pro-inflammatory cytokine which is involved in the Th1 response, and a role in plaque destabilisation has been suggested (68, 69). IL-18 is thought to exert its main pro-atherogenic effects by inducing interferon-γ production, which potentiates the inflammatory process and may lead to thinning or inhibition of the fibrous cap formation, resulting in vulnerable, rupture-prone plaques (57, 70). In addition, IL-18 increases the expression of matrix metalloproteinases in vascular cells and macrophages, which might also contribute to plaque destabilisation (57, 71). However, data regarding IL-18 as a potential predictor of acute cardiovascular events have so far been conflicting (72, 73).

4.2.2. The metabolic syndrome - a pro-inflammatory state

There is increasing evidence that the metabolic syndrome is associated with a chronic, low-grade inflammation (3). Several pro-inflammatory cytokines have been shown to be elevated in parallel with an increasing number of components of the syndrome, whereas the anti-inflammatory and adipocyte-specific substance adiponectin is consistently lower (74-77). Furthermore, pro-inflammatory cytokines have been reported to induce insulin resistance in both adipose tissue and muscle (78-80). Moreover, increased levels of CRP, IL-6 and low levels of adiponectin have been shown to predict the development of type 2 diabetes mellitus (81, 82). Recently, also IL-18 was reported to predict type 2 diabetes (83).

Some investigators have discussed that both type 2 diabetes, metabolic syndrome and atherosclerosis are multifactorial conditions which appear to have a common inflammatory
basis (84). And as both inflammation and the metabolic syndrome are known risk factors for CVD, it is currently discussed if a measure of inflammation should be included in the definition of the syndrome (3, 85).

So far, CRP has been the most likely candidate (85, 86). Cross-sectional and prospective studies have shown added prognostic information for cardiovascular risk stratification with CRP in populations with the metabolic syndrome (87-89). However, a recent prospective study showed that although both CRP and the metabolic syndrome were independent predictors of CVD, the combination of the two did not increase the predictive value (90). The role of other pro-inflammatory markers in predicting CVD in populations with the metabolic syndrome remains to be investigated.

In addition to a pro-inflammatory state, the metabolic syndrome is frequently accompanied by a hypercoagulable state with increased plasma coagulation and reduced fibrinolysis (91, 92). In particular, the main inhibitor of the fibrinolytic system, plasminogen activator inhibitor type-1 (PAI-1), has been consistently shown to be elevated in the metabolic syndrome (93). Thus, the metabolic syndrome can in part be considered both a pro-inflammatory and pro-thrombotic state (3).

4.2.3. Adipose tissue - an important source of inflammation

The classical perception of adipose tissue as a passive storage place of fatty acids has gradually been replaced by the notion of adipose tissue, and visceral fat in particular as an active endocrine organ. Visceral fat is now considered a central feature and potential cause of the metabolic syndrome (9), in part mediated by release of a large number of metabolically
active substances known as adipokines (Figure 4). Adipokines are involved in several biological processes, including inflammation, thrombosis, insulin sensitivity and energy balance (94). Not only adipocytes, but also stromal cells such as macrophages, fibroblasts and endothelial cells are involved in the production of various adipokines (95). Several pro-inflammatory markers have been shown to be produced in adipose tissue, including MCP-1, TNF-α, IL-1, IL-6, IL-8 and IL-18 (Figure 4) (96, 97). In the presence of obesity, adipose tissue produces cytokines in excess, whereas the production of adiponectin is diminished, thus shifting the balance to a pro-inflammatory state (98).

Whether systemic inflammation depends mostly on the quantity or the quality of adipose tissue is not known, but probably the quantity of visceral adipose tissue is important (95). In contrast to subcutaneous fat, visceral fat drains directly to the portal circulation, and a study in extremely obese patients indicated that visceral fat was the main contributor of plasma IL-6 levels (99). Thus, in obese people, it is likely that viscerally produced IL-6 drained via the portal circulation could be an important inductor of CRP-production in the liver (95, 96).

Moreover, IL-6 has been shown to induce PAI-1 production in visceral and subcutaneous adipose tissue (100). PAI-1 is produced in substantial amounts in human adipose tissue (101, 102), and in a study of obese patients abdominal visceral fat expressed 5-fold more PAI-1 than subcutaneous fat (103). Hence, adipose tissue is likely to be a major contributor to the pro-inflammatory, pro-thrombotic state characteristic of the metabolic syndrome.
4.3. Arterial stiffness and the metabolic syndrome

Changes in arterial elasticity are present throughout the atherosclerotic process, and are closely associated with endothelial dysfunction (104). Non-invasive measurements of arterial distensibility in various vascular segments may be performed by several methods, including pulse wave velocity or the inversely related pulse wave propagation time (PWP) (105). Such measurements of arterial stiffness have been shown to predict cardiovascular events in several studies. Most evidence comes from measurement of aortic pulse wave velocity in various populations (106-112), whereas one study reported predictive information of small artery elasticity in an asymptomatic population (113). Although measures of arterial stiffness may give prognostic information, it remains to demonstrate whether reducing arterial stiffness with treatment might reduce the risk of cardiovascular events (114). Aging affects all segments of the vasculature, and should be taken into account when arterial stiffness is evaluated in relation to the atherosclerotic process (114).

Arterial stiffness is a central feature in both type 1 and type 2 diabetes mellitus (115). In type 2 diabetes, the presence of micro- and macrovascular complications is associated with increase in arterial stiffness (116, 117). Furthermore, the age-related increase in arterial stiffness is steeper in individuals with type 2 diabetes than controls (118).

An increasing amount of evidence has also demonstrated increased arterial stiffness in subjects with the metabolic syndrome, associated with increasing number of components of the syndrome (119-123). Also in subjects with the metabolic syndrome, there seems to be a steeper age-related increase in arterial stiffness (119). Of note, individuals with improved metabolic status over time have experienced lower rates of arterial stiffness (122). Taken
together, arterial stiffness seems to be closely associated with the metabolic syndrome, and has been suggested to contribute to the increased cardiovascular risk in this population (115). Although low-grade inflammation is known to impair endothelial function (124), little is known about a potential mediating role of inflammation on the metabolic syndrome and diabetes-related arterial stiffness (115).

4.4. Non-pharmacological interventions

4.4.1. Preventive effects of life style modifications

Lifestyle modifications have been shown to reduce the risk of developing type 2 diabetes. In the Finnish Diabetes Prevention Study, 552 middle-aged, overweight patients with impaired glucose tolerance, 76% with the metabolic syndrome, were randomised to an intervention group (aimed at reducing weight, reducing fat intake, increasing fibre intake and increasing physical activity) or a control group. During 3.2 years of follow-up, the intervention group had a 58% reduction in the risk of developing diabetes (125, 126). Similar results were reported from the Diabetes Prevention Program in the United States, where lifestyle modifications (aiming at weight reduction and increased physical activity) reduced diabetes risk with 58% compared with controls (127).

Physical inactivity and reduced physical fitness have been identified as independent risk factors of CVD, and are also clustered with other risk factors (128-130). Also changes in physical fitness over time are associated with all-cause and cardiovascular mortality (131). In large prospective observational studies, increased physical activity has been associated with reduced all-cause and cardiovascular mortality (132, 133). Thus, the importance of physical activity is highlighted in international guidelines on primary and secondary prevention of
CVD (134, 135). Still, the guidelines are not clear concerning the necessary dosage of training, although even small doses seem to be preventive (136).

4.4.2. Prevention of cardiovascular events by omega-3 fatty acids and Mediterranean-like diet

Polyunsaturated fatty acids (PUFAs) are defined as fatty acids containing two or more double bonds, and are classified as n-3 or n-6 according to the location of the first double bond relative to the methyl end of the molecule (137). Increased intake of n-3 PUFAs from plants or fish is a central feature of the Mediterranean dietary pattern, which is also characterised by reduced intake of saturated fat and high intake of fresh fruit, vegetables and cereals (138). Mediterranean-like diet and n-3 PUFA supplementation have been reported to reduce the risk of cardiovascular morbidity and mortality (139). In the DART trial comprising 2033 patients with previous myocardial infarction, a 29% decrease in all-cause mortality was observed in men who were randomised to eat fatty fish twice a week compared to a control group (140). In the large GISSI secondary prevention trial, a 30% reduction in cardiovascular death was achieved with 1 g n-3 PUFA daily compared to the control group after 3.5 years of intervention (141). Also in the recent JELIS primary prevention trial, 1.8 g of eicosapentahanoic acid (EPA) daily in addition to statins reduced the risk of major coronary events by 19% compared to statins alone (142).

From large observational studies, adherence to a Mediterranean dietary pattern has been associated with reduced all-cause mortality and mortality from coronary heart disease (143, 144). In The Lyon Diet Heart Study, comprising 605 patients with a first myocardial
infarction, Mediterranean-style diet was associated with a 68% reduction in coronary events and deaths compared to Western-style diet (138).

4.4.3. Anti-inflammatory effect of non-pharmacological interventions

The mechanisms behind the protective effects of exercise, diet and n-3 PUFA supplementation are not fully explained. As inflammation seems to play a central role in the pathogenesis of metabolic syndrome, type 2 diabetes and atherosclerosis (85), it could be hypothesised that non-pharmacological interventions could have an anti-inflammatory effect.

It has been speculated that some of the beneficial effects of n-3 PUFAs might be due to a more favourable fatty acid profile in membrane phospholipids of inflammatory cells (145). However, results on inflammatory markers from previous intervention studies have been conflicting, depending both on the dose of n-3 PUFA supplementation and the variables studied (146). Also the effect on inflammation by various dietary strategies has been conflicting, and the interpretation of especially calorie-restricted diet interventions is complicated as weight loss itself might reduce inflammation (147). Although Mediterranean-like diet has been proposed to have a potentially anti-inflammatory effect (148), results from interventional studies have been conflicting (149, 150).

Epidemiological studies have shown that increasing levels of physical activity are associated with reduced inflammation in healthy subjects (151, 152). Furthermore, in uncontrolled trials of various study populations, it has been shown that exercise can reduce circulating markers of inflammation (153, 154). Still, only a few published controlled trials have evaluated the effect of exercise on markers of inflammation in populations with and without the metabolic
syndrome (155). As both endurance training (156, 157) and strength training (158) have been shown to reduce the amounts of visceral fat, it could be hypothesised that such exercise would have a beneficial effect on inflammation in obese people.
5. Aims of the thesis

5.1. Overall aim

To investigate the role of inflammation and atherosclerosis in the metabolic syndrome, and potential effects of non-pharmacological interventions on inflammatory markers.

5.2. Specific aims

5.2.1 To investigate the role of inflammatory markers as potential predictors of cardiovascular events in subjects with the metabolic syndrome (Paper II).

5.2.2 To investigate the effect of exercise on inflammatory markers in subjects with the metabolic syndrome, and a potential association with changes in adipose tissue compartments and components of the metabolic syndrome (Papers I and III).

5.2.3 To investigate the effect of Mediterranean-like diet and very long-chain n-3 PUFA supplementation on inflammatory markers in a population of elderly high-risk men, and a potential association with changes in anthropometric measures and fatty acid profile (Paper IV).

5.2.4 To investigate the impact of inflammation on metabolic syndrome-related arterial stiffness in elderly men (Paper V).
6. Material and methods

6.1. Study subjects and design

The Metabolic Syndrome and Anti-inflammatory effect of Training and Statin (SATS)-trial (paper I and III) took place at Nordland Hospital, Bodø in 2003. We studied 34 physically inactive men aged 20-75 years with the metabolic syndrome. Individuals with known coronary heart disease were excluded. Those treated with statins were either excluded, or statins were washed out for a period of 4 weeks before inclusion. The study subjects were recruited after advertisement in the local newspaper. The study had an unmasked randomised 2x2 factorial design. The participants were randomised into 1 of 4 groups: control (n=6), exercise (n=9), pravastatin (n=9) or the combination of both interventions (n=10). Data were obtained at randomisation and after 12 weeks. Two of the participants, one in the pravastatin group and one in the combination group were withdrawn from the study due to side effects. As the study was not performed on an intention-to-treat basis, data from these two participants were excluded from the statistical analysis. The study was carried out in compliance with the Helsinki Declaration and was approved by the Regional Ethics Committee. All subjects gave their written informed consent to participate.

In papers II, IV and V, we studied 563 elderly men age 64-76 years, 28% with known CVD, 34% smokers and 39% with the metabolic syndrome, and a large proportion taking a broad spectrum of medication. The basis for recruitment into the present study was a follow up of the participants from the Oslo Diet and Anti-smoking study carried out in 1972-1977, comprising 1232 men with high risk of CVD. The survivors of this population were in 1997 invited to participate in the Diet and Omega-3 Intervention Trial on Atherosclerosis (DOIT). In total, 910 survivors were contacted for screening, 655 attended the screening visit, and 563
were included. The study had a randomised 2x2 factorial design, and was placebo controlled for the n-3 PUFA capsules. The 563 participants were randomised into 1 of 4 groups: control group (n=142), dietary counselling and placebo capsules (n=139), n-3 PUFA capsules and no dietary counselling (n=140) and the combination of n-3 PUFA capsules and dietary counselling (n=142). Data were obtained at randomisation and after 36 months. After 3 years, 487 subjects completed the study and were included in the treatment effect analyses. Of the remaining, 38 had died, 29 dropped out because of disease states interfering with study follow-up, and 9 individuals were unwilling to complete the study. The study was carried out in compliance with the Helsinki Declaration and was approved by the Regional Ethics Committee. All subjects gave their written informed consent to participate.

6.2. Definition of the metabolic syndrome

In all papers, we have used the definition by ATP-III of the National Cholesterol Education Program (NCEP) (1, 3) (Table 1). It should be noted that in paper I, we used the definition from 2001, in which the cut-off for fasting blood glucose was ≥ 6.1 mmol/L, whereas in paper III also the 2005 version (using cut-off for glucose ≥ 5.6 mmol/L) was cited, thus the participants met the criteria for both definitions.

6.3. Intervention principles

In papers I and III, we studied the effect of exercise, pravastatin and the combination of both interventions. The exercise programme took place in a training studio three times a week, mostly in supervised groups. The duration of each workout was 45-60 minutes. Approximately 40% of the scheduled workout was endurance training and 60% was strength training of large muscle groups such as thighs, back and abdomen. The participants were
encouraged to record the trainings in logs. As an estimate of compliance, oxygen consumption was measured before and after the intervention period. Participants in the pravastatin groups received 40 mg pravastatin per day. The pravastatin intervention was not placebo-controlled. As an estimate of compliance, lipid profile was obtained before and after the intervention period.

In papers II, IV and V, we studied the effect of dietary counseling, very long-chain n-3 PUFA capsules and the combination of both interventions. The dietary counseling was undertaken on an individual basis and consisted of advice to increase the use of vegetable oil and margarines (rapeseed oil, olive oil and sunflower oil), vegetables, fruit and fish, and to decrease the use of meat and fat from animal sources. Special oil and margarine (VITA margarine; Norwegian Food Company Mills DA, Oslo) were specifically supplied to these participants at all visits. Overweight subjects were encouraged to adopt a calorie-restricted diet. As an estimate of compliance, food frequency questionnaires were recorded at baseline and 36 months. The dosage of n-3 PUFA supplementation was 2.4 g (4 capsules) daily, and the capsules (Picasol, Lube, Denmark) contained about 35% eicosapentaenoic acid (EPA; C20:5n-3), 20% docosahexaenoic acid (DHA; C22:6n-3) and 3.5 mg tocopherols/g to prevent fatty acid peroxidation. The placebo capsules (corn oil) contained 56% linoleic acid (18:2n-6), 32% oleic acid (18:1n-9), 10% palmitic acid (16:0) and 4 mg tocopherols/g.

6.4. Laboratory methods

All clinical data and laboratory analyses were obtained before randomization and after the intervention period. Blood samples were drawn after an overnight fast, and plasma and serum were frozen at –70°C to –80°C until analysis. Circulating levels of inflammatory markers and
adipokines in plasma and serum were measured by commercially available enzyme immunoassays, as described in details in the papers. In paper IV, fatty acid composition in serum phospholipids was analysed by gas-liquid chromatography in a random subset of participants (n=278).

In papers I and III, oxygen consumption was measured at 80% of estimated maximal heart rate, using the equation 220-age (years). Five recordings were made at the target frequency, and the median was used for statistical analyses. Quantification of subcutaneous and visceral fat compartments was performed by computerized tomography (CT). To minimize the amount of radiation, the CT scans were performed at only one site, corresponding to the disk between the 2nd and 3rd lumbar vertebrae, a level that has been shown to be closely correlated with visceral fat volume in men (159).

In paper V, arterial stiffness was evaluated by brachial PWP measured by a photoplethysmographic finger pulse-sensor (Medasonics, Mountain View, CA, USA) from the left third finger pulp, in fasting subjects and in a temperature-controlled room, as previously described (160). The photoplethysmography device evaluates the perfusion down to approximately 2 mm below the surface by combining an infrared source and a detector. PWP was defined by the time (milliseconds) from the initiation of the QRS-complex from ECG to digital pulse initiation from the finger pulp. The data were computerised and stored digitally, and the mean of three pulse wave registrations was obtained. Recordings were excluded if signals were poor or unreadable, or subjects had atrial fibrillation.
6.5. Statistics

As several biochemical markers were skewly distributed, non-parametric statistics were mainly used in papers I-IV, and detailed descriptions are given in the papers. In brief, differences between groups and differences in changes between groups were evaluated by Mann-Whitney U test, and Wilcoxon test was used to evaluate within group changes from baseline. Correlation analyses were performed using the Spearman method. In paper II, logistic regression analysis was used for calculation of adjusted odds ratios, and the chi squared linear by linear association was used for trend analyses. In papers III and V, Jonckheere-Terpstra test was used for trend analyses. In paper V, the main outcome variable (PWP) was normally distributed, and differences at baseline were evaluated with a t-test. Associations were analysed in a multiple linear regression model, and skewed data were log-transformed before entered into the model. A two-tailed significance level of 0.05 was used. The statistical analyses were performed with SPSS software, version 15.0 (SPSS Inc, Chicago, USA).
7. Summary of results

7.1. Paper I

We investigated the effect of a 12 weeks intervention with physical exercise on circulating markers of inflammation in 34 subjects with the metabolic syndrome. Pravastatin was used for comparison due to its documented anti-inflammatory effect. In the exercise group, there was a significant reduction in circulating levels of the chemokines MCP-1 (-33% vs. baseline, p=0.04 compared to no exercise) and IL-8 (-13% vs. baseline, p=0.007 vs. no exercise). Changes in MCP-1 were significantly correlated to changes in visceral fat (r=0.41, p=0.02). Our findings suggest that the protective effect of exercise might in part be due to suppression of the inflammatory process, and some of this effect might be mediated by a reduction in visceral fat.

7.2. Paper II

We investigated the role of inflammatory markers as potential predictors of cardiovascular events in subjects with and without the metabolic syndrome from the DOIT trial. The study comprised 563 elderly men with (n=221) and without (n=342) the metabolic syndrome. During 3 years, 68 cardiovascular events were recorded. In the total population, CRP, IL-18 and IL-6 were elevated in subjects with events (p<0.01). In subjects with the metabolic syndrome, IL-18 was the strongest predictor (adjusted odds ratio 2.9 [95% CI 1.1-7.8]), whereas in subjects without metabolic syndrome, only CRP appeared an independent predictor (3.3 [1.5-7.3]). There was a significant interaction between fasting glucose and both IL-18 (p=0.008) and IL-6 (p=0.024), but not CRP in the cardiovascular risk prediction. Elevated fasting glucose markedly increased the predictive power of inflammatory markers, and for IL-18, there was a stepwise increase in event rate by quartiles of fasting glucose.
Taken together, our findings suggest a mutually potentiating effect of hyperglycaemia and inflammation in the cardiovascular risk prediction.

7.3. Paper III

As IL-18 has been identified as a strong predictor of cardiovascular events in subjects with the metabolic syndrome, we investigated the effect of exercise on serum levels of IL-18 in the study from paper I. Levels of IL-18 were reduced by exercise only (p=0.036), pravastatin only (p=0.036) and the combination (p=0.017) vs. baseline, however without inter-group differences. Still, the reduction of IL-18 in the exercise groups was not negligible (17.5%), and the reduction of IL-18 was significantly associated with improvement of an increasing number of components of the metabolic syndrome (p for trend =0.034). This effect is likely to be caused by exercise, as this intervention improved several components of the syndrome compared to the control group (p=0.029). Our findings suggest that the protective effect of exercise might in part be due to reduced levels of IL-18 associated with improvement of the metabolic syndrome and its components.

7.4. Paper IV

Although Mediterranean-like diet and n-3 PUFA supplementation have been reported to reduce the risk of cardiovascular mortality and morbidity, the mechanisms are not fully clarified. We studied the effect of dietary counseling, very long-chain n-3 PUFA intervention and both on circulating levels of inflammatory markers in the DOIT trial. Levels of IL-18 were significantly decreased by diet (-10.5% vs. baseline, p=0.012 compared with no diet), and by n-3 PUFA supplementation (-9.9% vs. baseline, p=0.008 compared with placebo). Other measured inflammatory markers were not affected. Changes in IL-18 were significantly
but weakly correlated to changes in triglycerides ($r=0.20$, $p<0.001$), EPA ($r=-0.14$, $p=0.030$), DHA ($r=-0.14$, $p=0.034$), body mass index (BMI) ($r=0.16$, $p<0.001$) and waist circumference ($r=0.12$, $p=0.007$). Our findings suggest that the cardio-protective effects of Mediterranean-like diet and n-3 PUFA supplementation might in part be explained by reduced levels of IL-18, but probably beyond changes in serum fatty acids and body composition.

7.5. Paper V

Both arterial stiffness and inflammation have been shown to be associated with the metabolic syndrome and to predict cardiovascular events. As IL-18 seems to be particularly predictive of cardiovascular events in subjects with the metabolic syndrome, we aimed to investigate the influence of IL-18 and metabolic syndrome components on arterial stiffness in the DOIT population. At baseline, PWP was lower in subjects with the metabolic syndrome ($p<0.001$), reflecting increased arterial stiffness. Furthermore, an increasing number of metabolic syndrome components was significantly associated with lower PWP ($p$ for trend $<0.001$) and elevated levels of IL-18 ($p$ for trend $=0.002$). In a multivariate linear regression model, PWP was independently associated with IL-18 ($p=0.021$) and systolic blood pressure ($p<0.001$). Both arterial stiffness and IL-18 levels were improved by n-3 PUFA supplementation, as previously reported. Also changes in PWP after 36 months of intervention were independently associated with changes in IL-18 ($p=0.021$) and systolic blood pressure ($p<0.001$). The clinical importance of this clustering of metabolic syndrome components, inflammation and arterial stiffness, and the potential effect of n-3 PUFA supplementation warrant further investigation.
8. Discussion

8.1 Methodological considerations

8.1.1. General comments

It should be noted that we have only studied Caucasian male subjects. The DOIT study was an extension of the Oslo Diet and Anti-smoking study, so the population was already defined. This population is quite heterogenic, with a large range of morbidity and use of medication that might influence the inflammatory process. Furthermore, the subjects consist of long time survivors from a high-risk population, raising the possibility of survivor bias that should be taken into account when interpreting the prediction of events in paper II.

When planning the SATS study, we wanted the population to be as homogenous as possible to avoid too much biological variation to influence the intervention effect. However, we assumed quite wrongly that the metabolic phenotype with abdominal obesity and its related risk factors was a typical male problem. Indeed, it is now evident that the prevalence of metabolic syndrome is comparable in men and women, and that the syndrome probably contributes more to the relative cardiovascular risk in women (7, 32). Therefore, in retrospect, the SATS study should preferably have included also women.

Although the same definition of the metabolic syndrome has been used, the populations are quite different, and not necessarily generalisable to other cohorts with the metabolic syndrome. The SATS study comprised middle-aged men, with a high proportion of diabetics, whereas the DOIT study comprised elderly long-time survivors of men with long-standing hypercholesterolemia. Still, the age-adjusted prevalence of the metabolic syndrome in the DOIT study is comparable to that published in a large Norwegian cohort (17).
8.1.2. SATS study (Papers I and III)

The main limitation of the SATS study (paper I and III) is the small sample size, making the study especially vulnerable to type II statistical errors, and the study should be considered a pilot trial. While planning the study, we intended to perform a power analysis, which would have been appropriate. However, at this time point we could not identify any controlled study on the effect of exercise on inflammation (155). Thus, we were not certain about the effect estimate or which of the inflammatory markers that could be expected to change during the intervention period. As a practical solution, based on an uncontrolled study on the effect of exercise on circulating levels of adhesion molecules and MCP-1 in 12 subjects with heart failure (153), we aimed for including at least 30 participants, using the factorial design to increase the power in between-group analyses.

Another limitation of paper I and III is that all clinical examinations, including anthropometric measurements were performed by the principal investigator. Waist circumference in particular, is vulnerable to bias, and the results should be interpreted with caution. However, the analyses of inflammatory markers and measurement of adipose tissue compartments on CT were blinded, and the main result should therefore be reliable. In the correlation analysis in paper I (Figure 2), one outlier can be seen that was included in the analysis. Removing this outlier did not change the result. In paper III, another outlier can be seen (Figures 1 and 2), which was excluded from the analysis. Including this outlier did not change any of the results substantially, except that the reduction from baseline in the combination group was no longer statistically significant, whereas the significance persisted when analysing according to the factorial design.
8.1.3. DOIT study (Papers II and V)

Limitations regarding the study population are discussed above. In paper II, it should be noted that our results represent a post hoc analysis from an intervention trial, although we have tested for interactions and adjusted for intervention principles as appropriate. Furthermore, it should be noted that this study was not powered for clinical endpoints. However, registration of clinical endpoints was according to protocol. Finally, the limited number of endpoints may increase the risk of type II errors, especially in sub-group analyses. Thus, the main result with the strong association between IL-18 and end points should be reliable. It should also be noted that we have included a large number of covariates in the logistic regression analyses compared to the low number of endpoints, partly because the referees wanted us to include the classical risk factors, and partly as we had to adjust for the intervention principle. Recalculating odds ratios using only the strongest covariates did not change the main result.

The aims and objectives of paper V were considered important for penetrating potential mechanisms that could explain the obviously important role of IL-18 in the metabolic syndrome. Also in paper V, it should be noted that we have included a large number of covariates in the linear regression analysis compared to the size of the study cohort, but recalculating beta coefficients using only the strongest covariates did not change the main result.

8.2. General discussion

To simplify the discussion, paper II will be discussed first, followed by a common discussion of papers I and III, and finally papers IV and V.
8.2.1. Inflammatory markers and cardiovascular risk prediction in the metabolic syndrome

In paper II, we showed that IL-18 was a strong predictor of cardiovascular events in elderly men with the metabolic syndrome. As pointed out in the discussion, it is not appropriate to generalise this result to the general population, as our study comprises only elderly Caucasian men, and as survivor bias might be present. However, Espinola-Klein et al reported similar findings from a large cohort consisting of men and women (n=1263, mean age 62 years) with documented coronary artery disease, with IL-18 being the only independent predictor for cardiovascular mortality in subjects with the metabolic syndrome (161). To our knowledge, no other studies have to date investigated the combined impact of IL-18 and metabolic syndrome on cardiovascular events.

As pointed out in paper II, increased levels of CRP have been reported to add to the cardiovascular risk prediction of metabolic syndrome both in cross-sectional (87) and prospective studies (88, 89), whereas one prospective study could not find that the combination of elevated CRP and metabolic syndrome increased the predictive power (90). Langenberg et al investigated the combined impact of metabolic syndrome, adipokines and inflammatory markers on cardiovascular death in men and women during 20 years of follow-up (n=2118, age 40-95 years), and showed that IL-6 but not CRP remained a significant predictor when including both markers in multivariate analyses (162). In accordance with Espinola-Klein and Langenberg also we included all the inflammatory markers (IL-6, IL-18 and CRP) in the same model, but when excluding IL-18 from the model, also IL-6 (adjusted OR 2.7 [95% CI 1.1, 7.0]) and CRP (2.9 [1.1, 8.0]) became independent predictors in subjects with the metabolic syndrome. Thus, although CRP has been identified as the most consistent
inflammatory marker in predicting cardiovascular events (64, 73, 163, 164), this marker seems to disappear as an independent predictor in metabolic syndrome when including IL-18 and possibly IL-6 in the same model (161, 162). Hence, it seems like CRP is a more global marker of CVD, whereas IL-18, and possibly IL-6 seem to be more predictive in the presence of the metabolic syndrome. All studies taken together, inflammatory markers probably add to the CVD risk of the metabolic syndrome, but which of the markers that add most to the risk remains to be determined.

Another question is whether inflammation causes atherosclerosis or the other way around, or if other factors associated with the metabolic syndrome might cause both inflammation and CVD. As shown in Figure 4, systemic inflammation might be caused by both cytokines from the atherosclerotic plaque and other sources such as adipose tissue (41). The fact that CRP is downstream of the cytokine cascade from the atherosclerotic plaque could in part explain the robustness of this marker in predicting CVD. Furthermore, inflammatory markers are associated with other risk factors such as smoking, obesity, diabetes and hypertension (165). Thus, although inflammatory markers are highly predictive of cardiovascular events, it remains unknown whether inflammation is a causative factor, a marker of the rupture-prone plaque or if the association is explained by unknown confounding factors.

In our study, we found a significant interaction between fasting glucose and both IL-18 and IL-6, but not CRP in the cardiovascular risk prediction. Thus, a potentiating proatherogenic effect of hyperglycemia and inflammation could be a possibility, and perhaps different inflammatory markers are affected differently.
Experimental hyperglycemia has been shown to increase concentrations of various cytokines, including IL-18, in humans (166). Our results expand these findings by suggesting that hyperglycemia not only increases levels of IL-18, but also fuels the potential harmful effects of a given cytokine level. IL-18 can stimulate both type 1 helper T (Th1) and Th2 responses depending on its cytokine milieu and acts synergistically with IL-12 to stimulate a Th1 response with production of interferon gamma, a central feature in the atherosclerotic lesion (68). Recently, levels of IL-12 have been reported to be increased in subjects with type 2 diabetes and by experimental hyperglycemia (167, 168). As several pro-inflammatory markers were elevated in subjects with the metabolic syndrome, we speculate that our findings could in part be explained by a Th1 response mediated by IL-18 acting in synergy with a hyperglycemic pro-inflammatory milieu. Also other cells are likely to be involved, and it has been shown that the proatherogenic effects of CRP were potentiated by hyperglycemia, by increased expression of adhesion molecules and MCP-1 in endothelial cells (169).

8.2.2. Effects of exercise on markers of inflammation

In paper I, we showed that a combination of strength and endurance training had an anti-inflammatory effect by reducing plasma levels of the chemokines MCP-1 and IL-8. At the time of submission, we could not identify any other controlled study that showed a similar effect, and our small pilot study is still one of few controlled trials on the effect of exercise on inflammation (155). However, in uncontrolled studies on different populations, exercise has been shown to decrease levels of MCP-1, IL-8, CRP and TNF-α, and to increase adiponectin levels (153, 154, 170, 171). The last years, also some controlled trials have been performed, with conflicting results on circulating inflammatory markers in various populations (155, 172-175).
In paper III, we showed reduced serum levels of IL-18 by pravastatin, exercise and the combination vs. baseline, however not in between group comparisons. A recent publication reported reduced levels of CRP and IL-18 by exercise in obese subjects with type 2 diabetes (174). Our study expands these findings by showing a significant association between reduction of IL-18 levels and improved number of metabolic syndrome components, and by a potentially additive effect of pravastatin and exercise on the IL-18 reduction.

8.2.3. Effects of Mediterranean-like diet and very long-chain omega-3 fatty acids on markers of inflammation

In paper IV, we showed that both Mediterranean-like diet and very long chain n-3 PUFA supplementation for 36 months reduced serum levels of IL-18, whereas the other inflammatory markers and adipokines studied were not affected. It could be discussed if it is appropriate to highlight this finding, as multiple statistical tests were performed. However, in light of the results from other papers in this thesis and the existing literature, IL-18 seems to be a particularly important player, and the observed reduction fits with the overall picture. Furthermore, it seems more difficult to obtain an intervention effect in populations with frequent use of medication. Hence, no effect of Mediterranean-like diet on levels of CRP or fibrinogen in subjects under treatment for coronary heart disease has been reported (150), whereas another study showed beneficial effect of Mediterranean-like diet on levels of CRP, IL-6 and IL-18 in a medication-free population with the metabolic syndrome (149).

Previous reports on the effect of n-3 PUFA supplementation on markers of inflammation have been conflicting (146). Studies from our group have shown that although moderate doses (2.4 g/day) of n-3 PUFAs might have beneficial effect on circulating levels of adhesion molecules
(176), the contrary might be seen with higher doses (4.9-5.1 g/day) (177, 178). It should also be noted that n-3 PUFA supplementation in high doses (4 g daily) has been reported to transiently increase fasting glucose in patients with diabetes (179), whereas long term metabolic effects with comparable doses (3.4 d/day) have been shown to be safe in patients with coronary artery disease (180). Moreover, low to moderate doses (2 g daily) have been reported to improve insulin sensitivity in healthy individuals (181).

A reduction in serum levels of IL-18 by very long-chain n-3 PUFA supplementation has not been reported before, to the best of our knowledge. The fact that other inflammatory markers were not affected by this intervention with moderate doses of n-3 PUFAs (2.4 g daily) could in part be due to the frequent use of medication as mentioned above, but it could also be that n-3 PUFAs act differently on the various inflammatory markers. In contrast to most other cytokines, IL-18 is expressed constitutively in many cell types as a precursor, pro-IL18, which is inactive until cleaved by the enzyme caspase-1 (68). n-3 PUFAs might inhibit inflammatory gene expression via inhibition of nuclear factorκB, and recent reports suggest that this transcription factor is probably involved in the activation of caspase-1 (137, 182). Furthermore PUFAs, especially EPA and DHA, are natural ligands for peroxysome proliferator activated receptors (PPAR), which in activated state inhibit nuclear factor κB and thus several inflammatory processes (137, 183). Thus, both PPAR activation and inhibition of the nuclear factor κB pathway could contribute at least indirectly to the reduction of IL-18 levels by n-3 PUFAs.

Although not discussed in paper IV, in subgroup analysis of subjects with the metabolic syndrome, we observed a similar reduction of IL-18 levels by both interventions, however not
statistically significant. When classifying all study participants according to improvement of 0, 1, 2, 3, 4 or 5 components of the syndrome (defined as more than median decrease in triglycerides, systolic blood pressure, glucose and waist circumference, or increase in HDL, respectively), reduced levels of IL-18 were significantly associated with improvement of an increasing number of components, as discussed in paper V (Figure 5). In subgroup analysis, this association was still significant in subjects who underwent dietary intervention (p for trend <0.001), but not in subjects receiving n-3 PUFA supplementation.

Figure 5. Changes in serum levels of IL-18 by number of metabolic syndrome components that improved during the intervention period in the total population (p for trend < 0.001).
8.2.4. Additive effect of lifestyle interventions?

In papers III and IV, we showed a potentially additive effect of the intervention principles on levels of IL-18 (exercise and pravastatin, diet and n-3 PUFA supplementation, respectively). The combination of diet and exercise was not studied in this thesis. However, in paper III and IV, we showed a significant association between reduced levels of IL-18 and improvement of an increasing number of components of the metabolic syndrome by both exercise and diet intervention. Regardless of intervention principle, the question remains whether an improved metabolic control leads to reduced inflammation or the other way around, or if improvement of body composition has a beneficial effect both on inflammation and components of the metabolic syndrome. Moreover, it could be speculated that a non-intended change in diet occurred in the exercise groups in papers I and III. However, as dietary counselling was not part of the intervention, data about eating patterns were not recorded. Conversely, data about physical activity were not monitored in paper IV, although differences in physical activity between the groups are less likely, as the dietary intervention was on an individual basis.

Several intervention studies have examined the combined effect of diet and exercise intervention in obese individuals. Taken together, most studies show that a combined intervention reduces levels of several pro-inflammatory markers and increases adiponectin levels (155, 184-187), although some studies report effect of diet only with no additional effect of exercise (188, 189). On the other hand, a recent study showed that the combination of diet and exercise but not diet alone reduced several inflammatory markers, although the reduction in visceral fat was equal in all intervention groups (190). Thus, exercise could exert its effect through alternative mechanisms such as decreased expression of cytokines in muscle tissue or reduced chronic oxidative stress (191, 192).
Interestingly, a combined intervention with Mediterranean-like diet and increased physical activity maintained a weight reduction and decreased levels of CRP and IL-18 in obese women during two years follow up (193). Lifestyle interventions consisting of diet and exercise have been shown to improve several cardiovascular risk factors including the metabolic syndrome and to reduce the risk of developing type 2 diabetes (126, 194). Hence, current guidelines highlight the combination of increased physical activity (at least 30 minutes on most days of the week) and improved diet (decreased intake of saturated fat and simple carbohydrates, increased intake of fruits, vegetables, whole grain and fish) to achieve a sustained weight loss and reversal of the components of the syndrome (3). Our findings suggest that some of the protective effect of these lifestyle modifications might be due to decreased inflammation associated with improved body composition and components of the metabolic syndrome.

8.2.6. Arterial stiffness, inflammation and the metabolic syndrome

In paper V, we showed that arterial stiffness evaluated by PWP was more pronounced in subjects with the metabolic syndrome, associated with an increasing number of metabolic syndrome components and independently associated with IL-18. Although the metabolic syndrome has been shown to be associated with both arterial stiffness and a pro-inflammatory state, such a clustering of IL-18, arterial stiffness and metabolic syndrome has to our knowledge not been reported previously.

One crucial question is whether or not such a clustering has importance for cardiovascular risk prediction. A clustering of arterial stiffness, metabolic syndrome and inflammation measured by erythrocyte sedimentation rate was recently reported from a cohort of ischaemic
stroke patients (195). In paper II, we showed that IL-18 was particularly predictive of cardiovascular events in subjects with hyperglycemia. However, there was no significant association between fasting glucose and PWP, and increased arterial stiffness did not appear to increase the predictive power of IL-18 in the present study (data not shown). Although arterial stiffness has been shown to predict cardiovascular events in several studies, the most consistent evidence come from studies on aortic stiffness, measured by carotid-femoral pulse wave velocity (106-112, 195), whereas brachial PWP was measured in the DOIT study.

As already mentioned, it should be noted that we have studied an elderly population. Arterial stiffness is in part implicated in the normal ageing process (196), and as the metabolic syndrome is also more prevalent in elderly, the observed clustering could in part be explained by the age group of the study population.

The title of paper V indicates an independent association not only between arterial stiffness and IL-18, but also with components of the metabolic syndrome. Although not elaborated in the paper due to space constraints, there was an independent association between PWP and increasing number of metabolic syndrome components, also after adjustment for age, smoking, CVD, statins, ACE-inhibitors and IL-18. Among these components, only systolic blood pressure was independently associated with PWP, as previously reported (176).

The precise mechanisms of increased arterial stiffness in diabetes and metabolic syndrome are largely unknown. One of the main mechanisms thought to be involved is the formation of advanced glycation end products in the arterial wall, causing cross-linking of collagen, which may cause loss of arterial wall elasticity (197). Another proposed mechanism is that chronic
hyperglycemia and hyperinsulinemia might increase the activity of the renin-angiotensin-aldosterone axis, which might contribute to hypertrophy and fibrosis (198). Endothelial dysfunction might also contribute to arterial stiffening, in part due to decreased local availability of the endogenous vasodilator nitric oxide, and increased activity of vasoconstrictors such as endothelin-1 (199-201).

As low-grade inflammation impairs endothelial function (124), it has been proposed that inflammation might be an important contributor to arterial stiffness in the metabolic syndrome (115). Our findings suggest that this might be the case, and that IL-18 might be an important player: Adding other inflammatory markers and adipokines such as CRP, IL-6, PAI-1 and adiponectin to the regression model did not change the beta-coefficient of IL-18, whereas none of the other markers were independently associated with PWP (data not shown).

Interestingly, both arterial stiffness (160) and levels of IL-18 (paper IV) were improved by n-3 PUFA supplementation in this trial, whereas IL-18 but not arterial stiffness was improved by diet intervention. Changes in PWP after 36 months of intervention were independently associated with changes in IL-18, and there was a significant association between an increasing number of metabolic syndrome components that improved and decreasing IL-18 levels (Figure 5) and reduced arterial stiffness. However, the association between reduced IL-18 levels and improvement of metabolic syndrome components was only significant in subjects who underwent dietary intervention (p for trend <0.001), and not in subjects receiving n-3 PUFA supplementation, as discussed above. Also the association between
increased PWP and improvement of metabolic syndrome components was present in the diet group (p for trend =0.05), but not in the n-3 PUFA group.

Separate studies should be undertaken in different populations to assess the clinical importance of the clustering of arterial stiffness, inflammation and the metabolic syndrome, and the potential effects of treatment. Although diet intervention might explain the association with improvement of metabolic syndrome components, n-3 PUFA supplementation might be the most suitable intervention for effective treatment of both arterial stiffness and IL-18 levels.
9. Conclusions

1. Inflammatory markers and IL-18 in particular, strongly predict cardiovascular events in elderly men with the metabolic syndrome, and even more pronounced in the presence of elevated fasting glucose. Our results suggest a potentiating effect of inflammation and hyperglycemia in the cardiovascular risk prediction. This could have clinical importance for the risk stratification of subjects not only with the metabolic syndrome, but also with impaired fasting glucose and diabetes mellitus (paper II).

2. Exercise reduced levels of MCP-1, IL-8 (paper I) and IL-18 (paper III). The reduction of MCP-1 was associated with a reduction in visceral fat, whereas the IL-18 reduction was associated with an improvement in components of the metabolic syndrome. As inflammatory markers and IL-18 in particular, strongly predict cardiovascular events in patients with the metabolic syndrome, our results point to exercise as an important intervention principle in reducing cardiovascular risk in this population.

3. Mediterranean-like diet and very long-chain n-3 PUFA supplementation reduced serum levels of IL-18 in elderly high-risk men, whereas other inflammatory markers were not influenced (paper IV). Changes in IL-18 were significantly but weakly correlated with changes in body composition and serum fatty acids, and also associated with an improvement in components of the metabolic syndrome. Our results might explain some of the mechanisms behind the cardio-protective effect of these intervention principles, and point to Mediterranean-like diet and n-3 PUFA supplementation as important life-style interventions in high-risk individuals with and without the metabolic syndrome.
4. There was a tendency to additive effect of the intervention principles (pravastatin and exercise, diet and n-3 PUFA supplementation, respectively) regarding the reduction of IL-18 levels (papers III and IV), implying that a combination of these interventions could have a beneficial effect.

5. Arterial stiffness was more pronounced in subjects with the metabolic syndrome, associated with an increasing number of metabolic syndrome components and independently associated with IL-18 both cross-sectionally and longitudinally after 36 months of intervention (paper V). Our data suggest that IL-18 contributes to arterial stiffness in the metabolic syndrome. Hence, IL-18 and arterial stiffness might be potential therapeutic targets in this population.
10. Future perspectives

1. It remains to determine whether inflammatory markers and IL-18 in particular, are causative players or just markers of events in a population with the metabolic syndrome. A randomised controlled trial powered for clinical endpoints could answer this question. A combination of diet and exercise might be the most suitable intervention for reduction of IL-18 levels.

2. The potentiating effect of inflammation and elevated glucose in cardiovascular risk prediction remains to be elucidated. In vitro experiments with inflammatory cells being stimulated with IL-18 and IL-6 in the presence and absence of hyperglycemia, and with a range of readouts are currently ongoing, and could give valuable information about the mechanisms involved.

3. It remains to determine to what extent systemic inflammation and metabolic syndrome components are influenced by the quantity or activity of adipose tissue. Two studies are currently undergoing: One substudy from the DOIT cohort, comparing mRNA expression of inflammatory markers in adipose tissue from individuals with and without the metabolic syndrome, and a study of bariatric surgery patients in collaboration with Nordland Hospital, comparing the impact of quantity (on CT scan) and activity (mRNA expression) of visceral and subcutaneous adipose tissue compartments on systemic inflammation.

4. The clinical importance of the clustering of IL-18, arterial stiffness and metabolic syndrome remains to be elucidated. A randomised controlled trial powered for clinical endpoints could answer this question. An intervention with n-3 PUFA capsules might be the most suitable intervention for improvement of both arterial stiffness and IL-18 levels.
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