Dairy Products and Metabolic Effects

A Nordic Multicentre Study - Norwegian part

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Master Thesis in Clinical Nutrition
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>15:0</td>
<td>Pentadecanoic acid</td>
</tr>
<tr>
<td>17:0</td>
<td>Heptadecanoic acid</td>
</tr>
<tr>
<td>25-OH-D-vitamin</td>
<td>25-Hydroxyvitamin D</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>Apolipoprotein A-1</td>
</tr>
<tr>
<td>ApoB</td>
<td>Apolipoprotein B</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>C3</td>
<td>Complement factor 3</td>
</tr>
<tr>
<td>C4</td>
<td>Complement factor 4</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesterol ester transfer protein</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunoassays</td>
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<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
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<tr>
<td>HDL-C</td>
<td>High Density Lipoprotein Cholesterol</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>HOMA Index</td>
<td>Homeostasis model of assessment</td>
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<tr>
<td>Hs-CRP</td>
<td>High sensitive C-reactive protein</td>
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<tr>
<td>HSL</td>
<td>Hormone sensitive lipase</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
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<tr>
<td>LDL-C</td>
<td>Low Density Lipoprotein Cholesterol</td>
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<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
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<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acid(s)</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acid(s)</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program (American Guidelines)</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>Prostaglandin F2 alpha</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid(s)</td>
</tr>
<tr>
<td>SAT</td>
<td>Saturated fatty acid(s)</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride(s)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor-alpha</td>
</tr>
<tr>
<td>VCAM</td>
<td>Vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
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VLDL-C  Very Low Density Lipoprotein Cholesterol

vWF  von Willebrand Factor

WHO  World Health Organisation
1. Abstract

BACKGROUND: Some epidemiologic studies have suggested inverse relations between intake of dairy products and components of the metabolic syndrome.

OBJECTIVE: The objective was to investigate the effects of an increased intake of dairy products in persons, with a habitually low intake of dairy products and with traits of the metabolic syndrome, on body composition and factors related to the metabolic syndrome.

STUDY DESIGN: Middle-aged overweight subjects (n = 38) with traits of the metabolic syndrome were recruited at the Lipid Clinic, Rikshospitalet Oslo University and randomly assigned into milk or control groups. The milk group was instructed to consume at least 3 portions of low- to moderate-fat dairy products daily. The control group maintained their habitual diet. Clinical investigations were conducted at baseline and after the six months intervention period.

RESULTS: There were no significant differences between changes in body weight or body composition, blood pressure, markers of inflammation, adiponectin, or oxidative stress between the milk and the control groups. There was a significantly decrease in E-selectin, a marker of endothelial function in the milk group at the end of the study compared to the control group (P = 0.008). Among participants with a low calcium intake at baseline (<700 mg/d), there was a significant treatment effect for waist circumference (P = 0.023).

CONCLUSIONS: This study gives no clear support to the hypothesis that a moderately increased intake of dairy products beneficially affects aspects of the metabolic syndrome. The apparently positive effects on waist circumference in subjects with a low calcium intake suggest a possible threshold in relation to effects on body composition.
2. **Introduction**

2.1 **Master thesis as part of a multicentre study**

This master thesis is based on an intervention study undertaken at the Lipid Clinic, Rikshospitalet, Oslo University Hospital between January 2005 and July 2007. The study was part of a multi-centre Nordic study, with participating centres in Helsinki, Uppsala and Oslo. The results of the Nordic study as a whole were published in 2009 and is attached in full-text in this thesis for reference (Appendix 1).

2.2 **Metabolic syndrome**

2.2.1 **Definition**

The Metabolic Syndrome is defined as a clustering of cardiovascular risk factors; including (abdominal) obesity, hypertension, dyslipidaemia (elevated triglycerides and decreased HDL-cholesterol levels) and hyperglycaemia (impaired glucose metabolism) (1).

The concept of metabolic syndrome has existed since 1920, when the two Austrian physicians (Karl Hitzenberger and Martin Richter-Quittner) and the Spaniard Gregorio Marañón observed the relationship between blood pressure and diabetes mellitus in some of their patients. At about the same time, Kylin described the hypertension-hyperglycaemia-hyperuricaemia syndrome. In 1947, Vague drew attention to upper body adiposity as the obesity phenotype that was commonly associated with metabolic abnormalities in patients with type 2 diabetes and CVD. In 1988, Reaven described the "Syndrome X". He formed the hypothesis that insulin resistance is the common etiological factor for a group of disorders, consisting of impaired glucose tolerance, hyperinsulinaemia, dyslipidaemia and hypertension. The
metabolic syndrome has also been referred as the "deadly quarter" or the insulin resistance syndrome (2,3).

There are several different definitions of the MS, and the various cut-off for its components varies widely. The first official definition of MS was established in 1999 by the World Health Organisation (WHO). Later followed definitions defined by European Group for Study of Insulin Resistance (EGIR) (4) and the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) in 2001 (5).

The WHO and EGIR definitions both consider insulin resistance (or glucose intolerance) as an essential for diagnosis of MS. This is in contrast to the ATP III definition that regards insulin resistance as only one of several criteria. The WHO definition was developed for epidemiological purposes, while the ATP III definition was intended, due to its simplicity in use, for alerting clinicians to subjects at risk, and it is to date the most commonly used in clinical practice.

More recent definitions include those issued by the American Heart Association and National Heart, Lung, and Blood Institute (AHA/NHLBI) (5,6,6) and the International Diabetes Federation (IDF) (7). The AHA/NHLBI modified the ATP III by reducing the threshold for hyperglycemia and some other minor modifications. The IDF definition further modified the ATP III definition and aimed to unify the different definitions. The IDF definition considered central obesity (measured by waist circumference) as essential for diagnosis and was the first definition to include different cut-points of central obesity according to different ethnic groups. The threshold for waist circumference in the IDF definition was generally lower than in the preceding ATP III definition (the IDF definition required central obesity plus any two of four components: elevated triglycerides, low HDL-cholesterol, hypertension and elevated plasma glucose).

In 2009, Alberti K.G. et al. presented consensus criteria from the IDF and AHA/NHLBI which propose diagnosis of metabolic syndrome by using any 3 of 5
criteria (waist circumference, hypertension, increased plasma glucose, increased triglycerides and decreased HDL-cholesterol (8).
### Figure 1: Metabolic Syndrome definitions

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<tr>
<td>Glucose intolerance, IGT or diabetes and/ or insulin resistance* plus two or more of the following:</td>
<td>Insulin resistance (defined as hyperinsulinaemia – top 25% of fasting insulin values among the non-diabetic population)</td>
<td>Three or more of the following five risk factors:</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>≥ 6.1 mmol/l (110 mg/dl) but non-diabetic</td>
<td>≥ 5.6 mmol/l (100 mg/dl)*</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>≥ 140/90 mmHg</td>
<td>≥ 140/90 mmHg or treatment</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Raised plasma triglycerides:  ≥ 1.7 mmol/l (150 mg/dl) and/ or</td>
<td>&gt; 2.0 mmol/l (178 mg/dl) or treatment and/ or</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>Men: &lt; 0.9 mmol/l (35 mg/dl)</td>
<td>&lt; 1.0 mmol/l (39 mg/dl) or treatment</td>
</tr>
<tr>
<td></td>
<td>Women: &lt; 1.0 mmol/l (39 mg/dl)</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>Men: waist-hip ratio &gt; 0.90</td>
<td>Men: waist circumference ≥ 94 cm</td>
</tr>
<tr>
<td></td>
<td>Women: waist-hip ratio &gt; 0.85</td>
<td>Women: waist circumference ≥ 80 cm</td>
</tr>
<tr>
<td>and/ or BMI &gt; 30 kg/m²</td>
<td></td>
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<tr>
<td>Microalbuminuria</td>
<td>Urinary albumin excretion rate ≥ 20 µg/ min or albumin:creatinine ratio ≥ 30 mg/ g</td>
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*Insulin sensitivity measured under hyperinsulinaemic euglycaemic conditions, glucose uptake below lowest quartile for background population under investigation.

*The 2001 definition identified fasting plasma glucose of ≥ 6.1 mmol/l (110 mg/dl) as elevated. This was modified in 2004 to be ≥ 5.6 mmol/l (100 mg/dl), in accordance with the American Diabetes Association’s updated definition of impaired fasting glucose (IFG).

*Some male patients can develop multiple metabolic risk factors when the waist circumference is only marginally


2.2.2 Prevalence

Prevalence of metabolic syndrome worldwide

The prevalence of MS is increasing worldwide as is also the prevalence of obesity. Just as the prevalence of the individual components of the syndrome varies among populations, so does the prevalence of the metabolic syndrome itself (9). Differences in genetic background, diet, levels of physical activity, population age and sex structure, ethnicity and levels of over- and undernutrition all influence the prevalence. The prevalence also varies according to the definition of metabolic syndrome used (from 1 to 39%). Definitions with lower cut-offs for waist-circumference, for instance, will diagnose a larger group of people with metabolic syndrome.

Regardless of the differences in definitions and in the design of the studies that has investigated the prevalence certain inferences can be made (9). In all countries the prevalence of metabolic syndrome seems to increase with age, and there is wide variation in prevalence in both sexes (9). However, the prevalence seems to decline in the oldest population (10). Cultural factors are also important and metabolic syndrome is becoming more prevalent in developing countries in the Middle East (10).

For large scale screening purposes, NCEP/ATP classification is more practical than the WHO criteria, since determination of fasting glucose is sufficient and determination of insulin resistance levels is not required. In both definitions, the presence of type 2 diabetes does not exclude metabolic syndrome.

In European countries, the prevalence varies from 4–36% depending on age and definition of MS (10).

Hu G. et al (11) reviewed 11 European study cohorts and found that 15.7% of men and 14.2% of women had the metabolic syndrome as defined by the modified WHO definition (hyperinsulinaemia plus two or more other components including obesity, hypertension, dyslipidaemia, and impaired glucose regulation). When the syndrome was defined by the presence of two or more components without hyperinsulinaemia,
the prevalence was 35.3% for men and 29.9% for women; by the presence of three or more components without hyperinsulinaemia, it was 12.4% for men and 10.7% for women.

One third of middle-aged Americans were in 2004 found to have the metabolic syndrome as defined by the national Cholesterol Education Program (NCEP) (12).

Using NCEP criteria, the metabolic syndrome is detected in about 40% of the US population over 50 years. The diagnosis is most frequently driven by elevated blood pressure, followed by abdominal obesity and elevated fasting triglycerides (5).

According to census data from 2000, approximately 47 million Americans meet the diagnosis for metabolic syndrome, corresponding to about 40% of the adult population. This correlates with the 61% increase in the incidence of obesity between 1991 and 2000. It is worrisome that the increase in prevalence of the metabolic syndrome is higher in women than in men. In the NHANES III and NHANES 1999–2000 studies there was a statistically significant age adjusted increase in the prevalence of the metabolic syndrome in women, but not in men. Young women (20–39 years) had a 76% relative increase of prevalence, compared to a non significant increase of 5% in men in this age class (13). This is mainly driven by the constant rise in obesity in women, with presently 2 million more women than men being affected in the United States.

Prevalence of metabolic syndrome in Norway, Sweden and Finland

Little data exist on the prevalence of metabolic syndrome in Norway and the Nordic countries.

Hildrum et al. (14) performed a cross-sectional analysis of >10000 participants (aged 20–89 years of age) in a county of Northern Norway (HUNT 2) and found the prevalence of metabolic syndrome to be 29.6% using the IDF definition and a prevalence of 25.9% using the NCEP definition, and the prevalence increased strongly with age in both sexes. The most commonly fulfilled criteria were central
obesity (56.7% and 75% in men and women aged > 60 years, respectively) and hypertension (89.3% and 90.9%, respectively).

**Metabolic syndrome and risk of cardiovascular disease**

The metabolic syndrome is widely accepted as a risk factor for future type 2 diabetes and cardiovascular disease (15). It has been estimated that people with the metabolic syndrome are at approximately twice the risk of developing cardiovascular disease compared with those without the syndrome, and experience a five-fold increased risk of type 2 diabetes (16).

Gause-Nilsson I et al. (17) measured the prevalence of metabolic syndrome, as well as its individual components, in an elderly Swedish cohort (n=508), using the ATP III definition. 22.6% were found to have metabolic syndrome. The prevalence was higher in men (26.3%) than in women (19.2%). One third of the total sample had at least one of the five risk factors for metabolic syndrome. High blood pressure (> or =130/85 mmHg or use of antihypertensive/diuretic medication) was the most prevalent risk factor in both men (68.3%) and women (50.2%), while abdominal obesity was the overall second most common risk factor (27.2% of men and 42.7% of women).

Another Swedish study, by Welin L et al. in 2008 (18), found the prevalence of metabolic syndrome in middle-aged men and women to vary from 10 to 15.8% among the women, from 16.1 to 26% among 50-year-old men, and from 19.9 to 35% among the 60-year-old men. The prevalence of the metabolic syndrome varied with the definition used.

In a further study of middle-aged Swedes, the prevalence of metabolic syndrome defined by the ATP III definition was 14.8% among men and 15.3% among women, with an increase by age among women only (from 10% to 25%). Overweight was here also a dominant characteristic, defined by increased waist circumference (10).
Obesity is reported as the main cause of the rising prevalence of metabolic syndrome (5), and it could therefore be assumed that the prevalence of metabolic syndrome would also increase. Few studies have, however, explored this question.

Ilanne-Parikka P et al. (19) investigated in 2004 the prevalence of the metabolic syndrome by the modified WHO definition in a cross-sectional, population-based sample of 2,049 middle-aged individuals (aged 45-64 years) (FINRISK) and in 522 participants of the Finnish Diabetes Prevention Study (DPS). Metabolic syndrome was extremely common; 38.8% of the men and 22.2% of the women were diagnosed. Among women, the prevalence of the metabolic syndrome increased with increasing age. The high prevalence in men was mostly due to their high waist-to-hip ratio. The prevalence increased in both sexes with deterioration in glucose regulation.

2.2.3 Aetiology and pathophysiology of the metabolic syndrome

The causes of the metabolic syndrome are complex and have only been partially elucidated (3). The prevalence increases worldwide in accordance with the global increase in prevalence of obesity and diabetes (20).

The characteristics traits of many patients are overweight/obesity and a degree of insulin resistance, as well as older age (20). Although insulin resistance is not present in all patients (present in approx. 60-70 %), the most accepted hypothesis of the aetiology of the metabolic syndrome is insulin resistance (21), and abdominal obesity. Insulin resistance increases with increasing body fat mass. Insulin resistance, and its accomplice, hyperinsulinaemia, directly cause other metabolic risk factors. It is difficult to dissociate obesity and insulin resistance in patients with metabolic syndrome (1), by some, insulin resistance is considered to be at the core of the syndrome, while central obesity is its most prevalent clinical manifestation (3).

Abdominal obesity and insulin resistance

Overweight, characterised by an increased body mass index (BMI) is considered a risk factor for development of cardiovascular disease. However, central/abdominal
obesity is more strongly associated with increased risk of insulin resistance, than BMI per se, and thus associated with an increased risk of cardiovascular disease and diabetes. However, it is not known whether visceral fat causes insulin resistance or is simply associated with insulin resistance (21).

Abdominal obesity is defined as waist circumference or waist-to-hip ratio (22), and another particularly important anthropometric parameter has been increasingly applied in recent years is sagittal abdominal diameter (SAD) (22).

Insulin resistance is commonly used as the term for a state in which there is a defect in the response to the action of insulin (20). The consequence is an inability to maintain normal glucose homeostasis. Initially, the insulin-sensitive tissues (skeletal muscle, fat and liver) use insulin insufficiently. The pancreas then responds by secreting more insulin in an attempt to maintain normal blood glucose levels, and hyperinsulinaemia occurs. With this increase in plasma insulin levels, normal glucose levels are maintained. Eventually, however, insulin secretion declines resulting in both elevated blood glucose and insulin levels (21).

In obese individuals with excess intra-abdominal adipose tissue there is a tendency for increased concentration of non-esterified fatty acids (NEFA) in plasma.

Lipolysis of adipose tissue triglycerides is the major source of these NEFA. Insulin inhibits lipolysis and is therefore a major regulator of this process (21,23). The enzyme responsible for catalysing lipolysis is the cyclic AMP-dependent enzyme hormone sensitive lipase (HSL). A fall in insulin concentration stimulates activation of HSL, with resulting NEFA release in plasma.

NEFA are also derived through the lipolysis of triglyceride-rich lipoproteins in tissues by the action of lipoprotein lipase, another enzyme which is stimulated by insulin. Insulin resistance leads to less effective suppression of NEFA after meals and thus an increase in NEFA release into the bloodstream (21).
These NEFA are transported directly into the portal vein and thus to the liver (21). The flux to the liver may have direct metabolic effects, as the liver is overloaded with lipids which further enhance insulin resistance (1, 23).

Increased NEFA in plasma can also impair the ability of insulin to stimulate muscle glucose utilisation by the glucose-fatty acid cycle (23) and suppress hepatic glucose production (21).

NEFA derived from lipolysis of visceral fat are also delivered to skeletal muscle but it is unlikely that this is responsible for insulin resistance in skeletal muscle (21).

**Dyslipidaemia**

The MS is also associated with dyslipidaemia. There is usually an inverse relationship between plasma triglycerides (TG) and HDL-cholesterol concentrations, and an additional third component, the dyslipidaemia is therefore often characterised as the “lipid triad” – including high levels of TG, low levels of high density lipoprotein cholesterol (HDL) (21) and the appearance of small, dense low density lipoprotein particles (sdLDL) (24,25). Excessive postprandial lipaemia is also a common feature (24,25).

The dyslipidaemia is often associated with abdominal obesity (26), and the pathogenesis is linked to insulin resistance and the excess flux of NEFA in plasma and increased uptake by the liver (3,21).

Normally, insulin stimulates enzymes required for TG synthesis and reduces very low-density lipoprotein (VLDL) triglyceride and apolipoprotein B production and secretion (27).

However, in MS, the excess flux of NEFA to the liver may impair hepatic insulin action. This leads to increased triglyceride synthesis and storage, and excess secretion of triglycerides as VLDL in plasma (3,25,27).
High plasma concentrations of these triglyceride-rich VLDL particles stimulate cholesterol ester transfer protein (CETP), which promotes the transfer of TG from VLDL to HDL and a subsequent increased catabolism of HDL and thus decreased HDL concentrations (3,25). In addition, TG in HDL is a substrate for hepatic lipase that converts HDL to smaller, denser particles which have reduced antioxidant capacity (25).

The third component of the “lipid triad” associated with the metabolic syndrome is small dense LDL particles (sdLDL). The formation of these sdLDL is also closely associated with insulin resistance and hypertriglyceridemia (27), and the VLDL-triglyceride level is the major predictor of LDL size. The mechanism that leads to the formation of sdLDL is well elaborated. CETP facilitates the transfer of TG from VLDL to LDL in exchange for LDL cholesterol ester; hepatic lipase stimulates hydrolysis of the resulting triglyceride-rich LDL; and the increased lipolysis of triglyceride-rich LDL results in the formation of sdLDL (3,27). Thus, it seems that the presence of large triglyceride rich VLDL particles is a prerequisite for sdLDL formation, and such correlations have been observed, although sdLDL particle do occur in patients with only slightly raised or normal TG levels.

**Hypertension**

Hypertension affects as much as 85% of patients with the metabolic syndrome (2), and is often a consequence of obesity.

The sympathetic nervous system and renin-angiotensinaldosterone system (RAAS) is activated by insulin resistance and hyperinsulinaemia and this explains its relation to the MS (20).

Increased visceral fat accumulation is a strong predictor of arterial hypertension as it leads to overactivation of the sympathetic nervous system overactivation (3,20).

An increase in the sympathetic nervous system leads to sodium retention and volume expansion, endothelial dysfunction and alteration in renal function (2).
The local RAAS in the visceral adipose tissue exerts more powerful systemic effects compared with the subcutaneous adipose tissue (2). Activation of RAAS generates the production of angiotensin II and its pro-atherogenic effects, by inhibiting the vasodilatatory effects of insulin on blood vessels and glucose uptake into the skeletal muscle cells. There is a subsequent decrease in nitric oxide (NO) production in endothelial cells and vasoconstriction in smooth muscle cells, and inhibition of glucose transport (GLUT 4) in skeletal muscles (2). Insulin resistance also results in overactivity of angiotensin 1 receptor, which further leads to vasoconstriction and volume expansion.

It has also been suggested that chronic increases in portal venous fatty acid levels may be responsible for hypertension that accompanies visceral obesity, by mediating relative vasoconstriction (2,20).

**Inflammation, endothelial function and oxidative stress**

Chronic inflammation, procoagulation and impaired fibrinolysis are features of the metabolic syndrome, but is not included in the clinical definition of the syndrome (28). Chronic inflammation in metabolic syndrome is not accompanied by infection or signs of autoimmunity and no massive tissue injury seem to have taken place. The inflammatory activation in metabolic syndrome is therefore often referred to as “low-grade” chronic inflammation (29).

It is now widely accepted that adipose tissue excerts other roles than soley storage and release of NEFA. Adipose tissue secrete a number of adipokines or adipocytokines, which all affect insulin action (including leptin, adiponectin, TNF-α, IL-6 and CRP) (28), and adipose tissue in obese individuals secrete more of these molecules than adipose tissue in lean individuals. The inflammatory markers that have been linked to the metabolic syndrome include, C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), fibrinogen and interleukin-6 (IL-6).
In addition to these inflammatory markers, adipose tissue also secretes procoagulant proteins such as plasminogen activator inhibitor type-1 (PAI-1), tissue factor and factor VII (28).

The pathophysiology of inflammation in metabolic syndrome is not fully elucidated as yet. One theory is that the release of cytokines by the adipose tissue stimulates CRP production in the liver. Macrophage infiltration into adipose tissue in obese individuals can also explain the presence of increased levels of the inflammatory markers. Insulin resistance may also be causal of the higher cytokine production(28).

**C-reactive protein**

Chronically elevated levels of CRP are associated nearly all the main cardiovascular risk factors, including insulin resistance and diabetes, metabolic syndrome, hypertension, smoking, and dyslipidemia. A linear relationship between circulating levels of CRP and CVD risk has been demonstrated, and elevated CRP levels in obese individuals increase the risk of progression to type 2 diabetes mellitus (30).

However, whether CRP contributes to atherosclerosis or is a marker of risk is not known (28).

Elevated CRP levels associated with obesity, especially in subjects with abdominal obesity (31), and weight loss leads to a significant decrease in CRP levels. This results in a subsequent improvement in insulin resistance and thus demonstrates that there is a link between CRP levels and obesity (28,30).

Increased levels of CRP have been shown to cause induction of endothelial adhesion proteins VCAM-1, E-selectin, and angiotensin type 1 receptor. In addition, CRP activates induction of endothelial PAI-1, IL-6, TNF-α, and is thus associated with endothelial dysfunction (28,30).

**Tumor necrosis factor-α**

The secretion of TNF-α is increased in obese individuals. It is produced primarily from macrophages in obese adipose tissue (30). It has been suggested that this
cytokine mediates insulin resistance as it blocks the action of insulin (28). Weight loss decrease TNF-α and insulin resistance improves with TNF-α deficiency.

Results from animal studies suggest to the metabolic syndrome as TNF-α also seem to regulate plasma TG levels and glucose homeostasis in mice with targeted disruption of the TNF-α gene (28).

Circulating TNF-α may also contribute by its induction of CRP production and general systemic inflammation. In vitro experiments have also shown that TNF-α induces vascular adhesion molecules and cytokines, resulting in inflammatory and foam cell accumulation (30).

**Interleukin-6**

IL-6 is produced by a wide rage of cells, and as much as 30% is produced in adipose tissue. Elevated levels of IL-6 are positively correlated with obesity, insulin resistance and hypertension. Visceral adipose tissue has been shown to produce larger amounts of IL-6 than subcutaneous adipose tissue. Obese men typically have higher waist-to-hip ratios compared to women and may as a result experience greater metabolic effects from this pattern of adipose tissue deposition. This difference in fat distribution in men and women is emphasized in studies that have shown that in women with android obesity there is a similar relationship between elevated IL-6 and insulin resistance.

There is growing evidence that it also has roles in inducing lipolysis and decreasing appetite and weight gain (30).

IL-6 is a marker of increased cardiovascular disease, and has a role in hypertension by stimulating the central nervous system and the sympathetic nervous system (28).

IL-6 production induces CRP secretion, and there are data that suggest IL-6 decreases lipoprotein lipase activity, which results in increased macrophage uptake of lipids.
**Adiponectin**

Adiponectin is a plasma protein produced exclusively by adipose tissue (32). Physiologically, women seem to have higher adiponectin concentrations than men (33). Studies link hypoadiponectinaemia to the pathogenesis of obesity-related metabolic and vascular diseases. There is a significant negative correlation between BMI and plasma adiponectin levels in both men and women, and levels are negatively correlated with percent body fat, waist-to-hip ratio, and abdominal fat. Furthermore, adiponectin levels increase following weight loss. Low plasma levels are also associated with insulin resistance (22).

Adiponectin enhances energy consumption and fatty acid oxidation in the liver and muscle (Eckel et al 2005), which reduces the tissue triglyceride content, and consequently improves insulin sensitivity (22).

Adiponectin inhibits oxidative stress and inflammation (22), and is inversely associated with elevations in inflammatory markers. Low levels of adiponectin in obese women are associated with higher levels of CRP and IL-6 (28).

Adiponectin is a highly sensitive marker for the prediction of future cardiovascular events, and low levels are associated with progression of coronary artery calcification (Sutherland 2004). Adiponectin directly improves endothelial dysfunction by downregulating the expression of adhesion molecules on the endothelial cells (22).

**E-selectin**

E-selectin is suggested to be a marker of atherosclerotic activity associated with the metabolic syndrome (34). Some investigators have reported that levels of E-selectin correlate to total body fat and BMI but not to fat distribution in type 2 diabetes, and this seems to apply to subjects with the metabolic syndrome as well (34).

E-selectin levels may be regulated by adipocytokines such as TNF-a [12] or adiponectin (34).
Plasminogen activator inhibitor-1

The metabolic syndrome may also be related to the elevated levels of hemostatic factors like fibrinogen and PAI-1. PAI-1, an inhibitor of plasminogen activation which stabilizes fibrin, is disordered in obesity. High levels of PAI-1 have been associated with MI and CHD.

PAI-1 is synthesised and secreted by endothelial cells, mononuclear cells, hepatocytes, adipocytes and fibroblasts. It is regulated by the cytokines TNF-α and IL-6, among others.

PAI-1 is elevated in metabolic syndrome. Elevated PAI-1 levels have been shown to be associated with abdominal obesity and with BMI in men and women. PAI-1 appears to be related to the degree of obesity. For example, although insulin resistant, lean type 2 diabetics have been shown to have similar plasma PAI-1 levels compared to lean non-diabetic subjects (28). It is also correlated with other measures of obesity, including waist-to-hip ratio, reflecting abdominal fat, and with several metabolic factors, such as serum TG and insulin levels.

There is evidence suggesting that weight loss leads to a significant reduction in PAI-1 levels in obese subjects, due to decrease in blood lipids or insulin or reduced body weight. It has been shown that during weight reduction, the decrease in PAI-1 is more closely related to changes in adipose tissue mass than to changes in metabolic variables such as TG and insulin levels (28).

PAI-1 is a regulatory protein of the coagulation cascade, which is elevated in inflammatory and obese states as well as in the metabolic syndrome.

Obesity is also associated with increased circulating levels of the procoagulant factors tissue factor, fibrinogen, von Willebrand factor, and factor VII. Many of the circulating cytokines elevated in obese states also cause endothelial activation, resulting in low levels of platelet activation, prostaglandin secretion, and plug formation, and hypercoagulable state, which is thought to contribute to atherogenesis. This hypothesis has been validated by the demonstration that elevated levels of each
of these prothrombotic serum factors are associated with increased cardiovascular risks (30).

**Leptin**
Leptin is involved in the control of energy homeostasis and appetite regulator, and is one of the key vasoactive substances produced by adipocytes. During fasting, when plasma leptin levels decline, neural pathways in the hypothalamus cause the appetite to increase and energy expenditure to decrease as the body attempts to restore its fat stores (22). Leptin levels correlate with the amount of body fat; therefore, obese individuals have the highest levels of leptin, suggesting that these individuals leptin resistant as opposed to suffering from leptin deficiency. Leptin levels are physiologically higher in women than in men (33).

The potential effects of leptin in the pathophysiology of cardiovascular complications of obesity remain diverse. However, leptin is thought to contribute to insulin resistance and is considered to be one of the links between obesity, insulin resistance, and atherosclerosis. (22,30). There is a positive correlation between leptin and CRP and other inflammatory markers in healthy and obese subjects.

Evidence has suggested that leptin stimulates cholesterol uptake by macrophages, particularly in the presence of high glucose. This then triggers the formation of foam cells and the development of atheromatic lesions. Leptin may elevate the blood pressure by stimulating the autonomic nervous system. Obesity-related hypoadiponectinemia might also contribute to impaired endothelial function and overall proatherogenic effects (30).

It remains highly likely that all these markers of inflammation, endothelial function and oxidative stress are associated with the pathophysiology of the metabolic syndrome and may therefore be a potential target of prevention and treatment (30).
2.3 Metabolic syndrome and dairy products

2.3.1 Cardiovascular Disease

Cardiovascular disease (CVD), although decreasing in prevalence in the last decades, is still the major cause of death in Norway. The most important risk factors for developing cardiovascular disease include dyslipidaemia, overweight, hypertension, diabetes, smoking and inactivity, all of which are related to, and highly influenced by, lifestyle and diet. Although still prominent, the prevalence of these major risk factors such as hypertension, dyslipidaemia and smoking has also decreased in the past 25 years (35). In Norway there has simultaneously been a reduction in the intake of saturated fat in the (www.shdir.no). However, in this same time period the prevalence of obesity and diabetes has risen dramatically worldwide, Norway included. The reasons for this development is multifactorial (35), however, there is a clear link between the development of obesity and an increasingly sedentary lifestyle and an increase in calorie consumption (mainly due to an increase in simple carbohydrates).

2.3.2 Metabolic syndrome and dietary recommendations

The Metabolic Syndrome is as outlined earlier a clustering of the risk factors for CVD and thus diet is of major importance. There is to date, however, no consensus regarding specific nutrient recommendations for this syndrome. However, there is an agreement that energy restriction, regardless of dietary macronutrient composition, is beneficial for weight reduction, which consequently leads to an improvement in the metabolic risk factors (36). General national dietary recommendations in large parts of the world suggest lowering the total intake of fat; specifically saturated fat and trans fat intake as a means to improve the overall health of the population and to prevent cardiovascular disease. The same dietary principles apply for secondary prevention of CVD (5), in addition to specific recommendations for dietary cholesterol intake.
These general recommendations have been based on the assumption that saturated fat intake is associated with risk of accelerated development of cardiovascular disease and premature mortality from heart attack and stroke (37).

2.3.3 Dairy food consumption in Norway

Norway has always had a relatively high intake of milk and other dairy products. As much as 75% of the Norwegian population is milk consumers, and of the remaining 25% who does not drink milk, all are most likely consumers of other dairy products, such as either cheese or yoghurt. The intake of milk has decreased in the last 30 years, while yoghurt and cheese consumption has been on the increase over the past years. Data from 2008 and 2009 also show a slight decline in the consumption of cheese. Consumer data from 2009 reveals that the intake of milk was 97.3 litres per person, cheese and yoghurt intake per person was 17.3 kilos and 9.7 litres, respectively. About 82% of the total milk consumed in Norway, consists of low-fat products (0.1-1.5% fat). The neighbouring countries Sweden and Finland both have higher total intakes of milk, cheese and yoghurt compared to Norway (www.melk.no).

Norwegians have a positive attitude towards dairy products; as surveys have shown that 8 out of 10 consider milk as a healthy food product. Six out of 10 are positive to milk drinking, men being significantly more positive than women (www.melk.no).

Dairy products provides approximately 70% of the total daily dietary calcium intake in Norway, but as the overall consumption of dairy has decreased the calcium intake now only corresponds to 61% of the amount consumed in 1980 (www.melk.no).

2.3.4 Dairy products and cardiovascular disease

Milk fat is a major source of saturated fatty acids (38). Strong associations between intake of dairy fat and coronary heart disease have been indicated in ecologic studies (39-41), whereas prospective cohort studies have shown a more mixed picture (reviewed in (42).
Some studies have documented a positive correlation between the intake of dairy products and CHD mortality (43, 44) and shown that high-fat dairy products compared with low-fat dairy products are associated with an increased risk of CHD (45). Other studies are inconclusive and do not support a correlation between the intake of dairy products and CHD mortality (46-50).

Elwood et al published a review article in 2004 (51), reviewing 10 prospective cohort studies and 2 case-control studies on dairy products and CHD. All but one study in this review suggested a reduced risk of ischemic heart disease and ischemic stroke in subjects with the highest intake of dairy products. This review mainly looked at full-fat dairy products as they were the most commonly used products at the time when the studies were undertaken.

Moss and Freed (52) observed that consumption of whole milk was significantly correlated with CHD in 15 European countries. There was, however, a slightly negative correlation between CHD and cheese and fermented milk products. The result of this observational study is though limited due to only describing food consumption using food balance sheets.

### 2.3.5 Dairy products and metabolic syndrome

Dairy products are an important part of the Western diet as a source of good-quality protein and several vitamins and minerals. They may have positive as well as negative health effects (38, 53). Some epidemiologic studies have suggested an inverse relation between intake of dairy products and components and prevalence of the metabolic syndrome (54-56), which has not been apparent in other studies (57).

Some of the positive effects of dairy products are related to the metabolic syndrome and its different components. These positive findings are most certainly ascribed to various dairy products and its different constituents. Dairy products contain many different nutrients which may have various effects and characteristics that counterbalance the commonly expected negative effects.
The consumption of dairy products has been inversely associated with the prevalence and incidence of the metabolic syndrome in a number of epidemiological studies (54,55,58,59).

The cross-sectional study by Mennen et al (59) showed that consumption of dairy products were inversely related with the prevalence of the metabolic syndrome in men, but not in women. Men who consumed more than 1 portion of dairy/ day had a 40% lower prevalence of the metabolic syndrome compared with men who did not consume dairy products at all. Furthermore, Azadbakht et al (54) demonstrated an inverse association between dairy product consumption and the metabolic syndrome in Tehranian subjects aged 18-74 years. The subjects in the highest quartile of dairy consumption (≥3.1 servings/day of milk, yoghurt, cheese) had lower odds of having the metabolic syndrome compared with subjects in the lowest quartile of dairy consumption (<1.7 servings/day). In addition, Elwood et al. (58) have, in the Caerphilly study, found a negative relationship between milk and dairy product intake and the prevalence of metabolic syndrome in a study among adult men.

The Coronary Artery Risk Development In Young Adults (CARDIA) study, found an inverse association between the frequency of dairy intake and 10 year incidence of the development of metabolic syndrome, among more than 3000 young overweight men and women (55).

However, which of the components in dairy products that may be responsible for these positive effects is not clear, neither are the mechanisms behind these effects. However, several intervention studies have been performed on the effects of single nutrients from dairy products on the different features of the metabolic syndrome. In this respect, much attention has been paid to calcium, protein and fat (60).

**BODY COMPOSITION**

The consumption of dairy foods and calcium in dairy foods has been suggested to be beneficial in the regulation of body weight (61-63), especially in observational studies (64). Results from intervention studies, on the other hand, are inconsistent (61,65).
It has also been indicated that milk consumption and intake of dairy proteins are inversely related to the risk of hypertension (66-68), and intervention studies have shown a blood pressure–lowering effect of milk products and milk peptides (56,69).

An intervention study has shown that weight loss with milk supplementation induced a smaller increase in desire to eat and hunger compared to the control group. The effect in this study suggests that milk supplementation, due to the protein content of milk, attenuates the orexigenic effect of body weight loss (70).

A further randomized intervention study by Zemel et al (71) found that subjects on a weight maintenance diet who had a daily intake of ≥ 3 portions of dairy products exhibited greater fat oxidation and was able to consume greater energy without greater weight gain compared to the low dairy group (<1 portion of dairy/day).

**BLOOD PRESSURE**

The Dietary Approaches to Stop Hypertension (DASH) trial in 133 hypertensive American men and women (72) showed that an 8-week diet including low fat dairy products decreased blood pressure more than a diet high in fruit and vegetables.

Alonso et al (73) evaluated a prospective cohort study, and found that higher low-fat milk intake (≥3 portions/ day versus <1 portion/day) was associated with lower increases in systolic blood pressure in non-hypertensive whites men and women, but not in African Americans.

A study by Engberink et al. (74) on elderly Dutch subjects (> 55 years of age) in the Rotterdam study observed that low-fat dairy intake may contribute to prevention of hypertension in older age (20 % risk reduction after 6 years). The subjects were non-hypertensive at baseline and blood pressure was assessed again after 2 and 6 years.

Wang et al (75) also confirm the observation that an intake of low-fat dairy products is inversely associated with risk of hypertension in middle-aged and older women (> 45 years of age). In this prospective cohort of 28,886 US women aged >or=45 years, subjects with hypertension (n=8710) were identified from annual follow-up.
questionnaires during 10 years of follow-up and intake of dairy products at baseline were assessed from semi-quantitative food frequency questionnaires. The risk of hypertension decreased in the higher quintiles of dietary calcium. Adjustments for dietary calcium significantly attenuated the inverse association of low-fat dairy intake with risk of hypertension.

In a prospective cohort of 28,886 US women aged 45 years followed over a period of 10 years, a high intake of low fat dairy products (top vs. lowest quintile) was associated with a significant 11% reduction in the risk of developing hypertension (76). Interestingly, this reduction in risk was also significant across quintiles of dietary calcium and dietary vitamin D, but was not across quintiles of calcium or vitamin D supplements.

**INFLAMMATION**

However, the relationship between dairy consumption and the chronic inflammation linked to the metabolic syndrome has not yet been studied in depth (77).

Zemel et al (78) evaluated the acute effects of a dairy-rich diet on oxidative and inflammatory stress in overweight and obese subjects in a small cross-over study.

A dairy-supplemented diet was compared with soy-supplemented eucaloric diets. The dairy-supplemented diet resulted in significant suppression of oxidative stress (8-isoprostane-F(2alpha) and lower inflammatory markers (tumor necrosis factor-alpha) and increased adiponectin, whereas the soy exerted no significant effect.

Van Meijl et al (79) investigated the effects of low-fat dairy consumption on inflammatory markers and adhesion molecules in overweight and obese subjects in an intervention study. Results showed a non-significant decrease in TNF-α and a non-significant increase in TNF-α receptor-1 (s-TNFR-1) after a period of low-fat dairy intake (500 ml low-fat milk and 150 g low-fat yogurt). There were no other effects on other markers of chronic inflammation and endothelial function.
An intervention study investigating high-dairy eu caloric diet and hypocaloric diet in overweight men and women has shown clinically significant reductions in plasma CRP (11% and 29% respectively) and increase in plasma adiponectin concentrations (8% and 18% respectively) (80). These data suggested that dietary calcium may suppress adipose tissue oxidative and inflammatory stress associated with obesity. They also emphasized that dairy foods may beneficially alter circulating CRP and adiponectin levels independently of changes in body weight. However, the extent to which these mechanisms per se underlie some of the apparent cardio protective properties of dairy foods remains to be more thoroughly demonstrated.

2.3.6 Metabolic syndrome and effects of dairy food components and individual dairy foods

The most commonly associated component of dairy food is that of dietary calcium. Even though the effects of dairy products may be a result of the synergy between individual components, each nutrient and compound has a biological function of its own (81).

**MILK FAT**

Milk fat is associated with increased cholesterol levels due to its high content of saturated fatty acids. The main cholesterol-increasing saturated fatty acids are palmitic acid and myristic acid (16:0 and 14:0). These fatty acids are known to down regulate LDL receptor formation. The medium-chain fatty acids (8:0 and 10:0) may affect the cholesterol in a similar way (82).

The association between intake of saturated fatty acids and increased cholesterol concentrations is well documented (83,84), and saturated fatty acids have been shown to impair insulin sensitivity when substituted for unsaturated fatty acids (85,86).

For this reason the general national dietary recommendations often recommend low-fat dairy products as part of a healthy diet to reduce the intake of saturated fat.
**TRANS FAT**

Trans-fatty acids naturally occur in dairy products, with vaccenic acid (18:1 trans-11) being the most abundant. It is known that the intake of trans-fatty acids is strongly related to the development of CVDs. It is also known that trans-fatty acids increase LDL-cholesterol, triglyceride concentrations, and Lp(a) and affect prostaglandin balance and thereby thromogenesis, all with an impact on the development of CVD (42). Natural trans-fatty acids may be less atherogenic than industrially produced trans-fatty acids; however, this is debatable. The content of trans-fatty acids in milk fat is normally between 3% and 6%. The food matrix may have an impact and there may be a threshold level for the effect of trans-fatty acids to be seen (36). The TRANSFACT study was a randomized, controlled, crossover study investigating the effects of industrially compared to natural trans-fatty acids (about 5% of daily energy). The study showed that industrially produced trans-fatty acids resulted in lower plasma HDL-cholesterol concentrations in men, compared to trans-fatty acids from natural sources (87).

**Saturated fats**

Dairy products provide a source of dietary saturated fatty acids. Generally, saturated fatty acids have been reported in the literature to increase LDL-cholesterol (83), a risk factor for CHD (88). However, recent evidence suggests that perhaps the saturated fat within dairy products may not have the same health effects as other saturated fatty acids (89).

Saturated fatty acids from dairy products have been demonstrated to have a neutral effect on cholesterol, may increase in HDL-cholesterol, or a give a more favourable LDL profile overall. One cross-sectional study of 291 healthy adult males found an association between milk fatty acid intake and a reduction in small LDL-cholesterol particles reported by (90).

The saturated fatty acids in milk fat include shorter and medium chain fatty acids (2:0–10:0), lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), and stearic...
acid (18:0). Other fatty acids in milk fat are oleic acid (18:1) and linoleic acid (18:2n-6). The longer chained fatty acids, lauric, myristic, and palmitic acids are all cholesterol elevating fatty acids and it is possible that myristic acid is the most cholesterol elevating fatty acid. Stearic acid is, however, different from the other longer chained fatty acids present in dairy fat since it may have neutral effects on cholesterol level (83). The proportion of stearic acid in milk fat is about 11% (36).

**Total dairy fats**

In the Hoorn study, however, low-fat dairy consumption was positively related, while high-fat dairy consumption was inversely related to risk factors of the metabolic syndrome (57). Another study (91) of 62 elderly males also found an inverse relationship between milk fat intake and BMI, waist circumference, fasting plasma glucose, and LDL–HDL ratio. These paradoxical findings suggest the effect may lay in the food, not primarily the saturated fat component. It also demands research on the whole dietary composition (and lifestyle) contexts in which milk fat is consumed.

**FERMENTED PRODUCTS**

Some studies have indicated that dairy products may have hypocholesterolemic effects in humans, especially skim milk and various fermented dairy products (92,93).

A possible mechanism for the moderate cholesterol-decreasing effects of fermented milk products is that the intestinal bacteria bind bile acids to cholesterol, resulting in an excretion of bile acid-cholesterol in the faeces. The colonic fermentation of indigestible carbohydrates and the production of short-chain fatty acids is suggested to affect systemic lipid metabolism as well (94).

Some bacterial strains have the ability to produce bioactive peptides (94). ACE inhibitory peptides present in fermented milk products (94) are not degraded by enzymes in the intestine, but are absorbed directly and can inhibit ACE in the aorta, which may partly explain the antihypertensive effect of fermented milks (94-96).
CHEESE

There is evidence suggesting that different dairy products may have very different effects on components of the metabolic syndrome.

Butter has a higher concentration of milk fat than any other dairy product (94). Several human metabolic studies have documented the hypercholesterolaemic effect of butter (94).

Controlled studies have demonstrated a cholesterolaemic effect of milk comparable to that of butter, whereas the possible effects on other CHD risk markers have not been fully elucidated (94).

Several observational studies do not indicate a non-beneficial effect of cheese with regard to CHD risk (37,39,94,97).

Well-controlled human studies have demonstrated that cheese does not increase plasma cholesterol to the same extent, but rather has a smaller effect in raising LDL cholesterol in comparison to butter and even milk (98-100). Cheese intake was inversely related to a first myocardial infarction in a Norwegian study (101), but in a Costa-Rican study, higher cheese consumption was associated with increased risk of myocardial infarction (36). Until now, only the effects of regular hard cheese have been investigated, therefore the results can therefore not be extrapolated to other types of cheese (94). Despite this, analyses of cross-sectional data have suggested that cheese consumption may result in a greater risk of obesity and the metabolic syndrome (102).

This difference between butter and cheese may be explained by the unique physical structure of cheese, in which the fat content is encapsulated within a casein structure (36).

Cheese also contains a large variety of bioactive peptides including angiotension-converting enzyme (ACE), which may have antihypertensive effects (36).
2.3.7 POSSIBLE MECHANISMS OF EFFECT OF DAIRY FOODS AND ITS COMPONENTS

Mechanisms of calcium on lipid profile
Calcium may affect the serum lipid profile, and two potential mechanisms have been proposed. Firstly, calcium may inhibit fat absorption in the intestine. Calcium interacts in particular with saturated fatty acids to form Ca-fatty acid soaps and the formation of these insoluble complexes increases faecal fat excretion (60). The reduced absorption of saturated fatty acids may contribute to the LDL-cholesterol-lowering effects of calcium. Another possible mechanism is that calcium binds to bile acids, thus increasing its secretion, and inhibiting bile acids reabsorption into the enterohepatic circulation. This consequently lead to an increase in the conversion of cholesterol to bile acids and ultimately decreased LDL cholesterol levels (60).

There seems to be a difference between the effects of dairy and supplemental calcium, however, which may be due to the difference in the chemical form (calcium phosphate in dairy products vs. calcium carbonate in supplements) or due to the synergistic action of other dairy components (60).

Mechanism of calcium on body composition
Calcium may also be responsible for mediating body weight and fat mass (103). It has been proposed that the effects of calcium on body weight are only present in populations with low habitual intakes, and that at calcium consumption above 800 mg per day, no additive beneficial effects of increasing dietary calcium will occur. It might also be speculated that calcium reduce body weight and fat mass only when part of an energy-restricted diet. However, this is not supported by the all studies.

For the effects of calcium on body weight and body composition, the ability of calcium to bind to fatty acids and thereby inhibit fat absorption is proposed as one possible mechanism of action. Another way by which ca might affect body composition is by regulating intracellular calcium levels, as hypothesised by Zemel et
al (104). Intracellular calcium levels are regulated by parathyroid hormone and 1,25-hydroxyvitamin D (calcitriol). High dietary Ca depresses the levels of calcitriol, thereby decreasing intracellular calcium. This results in a stimulation of lipolysis. Additionally, low intracellular Ca inhibits the expression of fatty acid synthase, which is a key enzyme in de novo lipogenesis (104). Therefore, calcium intake may directly affect the storage and breakdown of fat in adipose tissue.

**Calcium and blood pressure**

Several potential mechanisms may explain the positive effect of calcium on blood pressure, including reduced membrane permeability to monovalent and divalent cations, reduced intracellular Ca levels, decreased concentrations of calcium regulating hormones, reduced sympathetic nervous system activity, and altered metabolism of other electrolytes, for example, increased sodium excretion (60). Again the effect of calcium might be mediated by suppression of the hormone calcitriol. Suppression of this hormone could lower intracellular Ca levels in vascular smooth muscle cells, thereby reducing peripheral resistance and blood pressure (105).
3. **Aims of the study**

The aim of this study is to investigate the effect of an increased intake of dairy products, on markers of the metabolic syndrome, in individuals with low habitual dairy food consumption and traits of the metabolic syndrome.

The hypothesis is that foods containing more dairy fat (and thus a higher proportion of short and medium chain fatty acids, calcium and possibly other important nutrients) favourably affect energy balance, appetite and the metabolic profile in subjects prone to develop abdominal adiposity and metabolic syndrome. Some of the main endpoints include effects on body weight and abdominal fat (abdominal obesity), lipid profile and inflammatory markers. It is also of interest to investigate whether the increased intake of dairy products change the composition of the participants’ diet as a whole, in terms of food choices and nutrient content.

There are few interventions studies that have investigated the effects of an increased intake of dairy products on the metabolic syndrome. However, a prospective American study (the Cardia Study) found that overweight adolescents who consumed dairy products to develop fewer metabolic abnormalities characteristic for the metabolic syndrome, than those who did not (55). Findings from a Norwegian study showed that subjects free from myocardial infarction had higher proportions of fatty acids derived from dairy fat in their adipose tissue than those who developed myocardial infarction (106). An earlier study based on a North-American population has shown positive effects of dairy products on risk factors for metabolic syndrome (107). It is now of interest to explore whether an intervention study on a Norwegian population can verify results from these earlier studies.
4. Subjects and Methods

4.1 Subjects

This study was conducted at the Lipid Clinic, Rikshospitalet, Oslo University Hospital between January 2005 and July 2007. This study is part of a Nordic multicentre study undertaken simultaneously in Norway, at the University of Helsinki, Finland and Uppsala University, Sweden. Participants were recruited and followed up at each centre, and the results were collated, analysed together and published collectively in the American Journal of Clinical Nutrition in 2009. For the purpose of this thesis, the Norwegian part and data are presented only.

Apparently healthy men and women aged 30-65 years were recruited through advertisements in local media (newspaper and tele-text), and through personal contacts with subjects from the patient pool at the Lipid Clinic.

4.1.1 Inclusion criteria

Inclusion criteria were: limited habitual intake of dairy products (≤ 2 portions/ day; 1 portion defined as ≤ 200g milk (sum of milk, cultural milk and/or yoghurt), 40g cheese or 10g butter/day)). Dairy intake was evaluated using dietary questionnaire and/or interview. The other main inclusion criteria was traits of the metabolic syndrome (i.e. the subjects had to fulfil two or more of the criteria for metabolic syndrome according to National Cholesterol Education Program Expert Panel (NCEP): fasting plasma glucose ≥ 6.1 mmol/l, serum triglycerides ≥ 1.7 mmol/l, serum HDL cholesterol < 1.0 mmol/l (40 mg/dl) (men) and < 1.3 mmol/l (50 mg/dl) (women), blood pressure ≥130/ 85 mmHg and waist circumference >94cm (men) and >88cm (women).
4.1.2 Exclusion criteria

Individuals were excluded if they had BMI > 36.1 kg/m², known type 1 diabetes, or treated type 2 diabetes, glycated haemoglobin (HbA1c) \( \geq 7.5\% \), known abnormal thyroid hormone levels, or high thyroid stimulating hormone (TSH) level and blood pressure >160/100 mmHg.

Also excluded were individuals taking lipid lowering drugs (fibrate, statin), those treated with Cyclosporin A, oral anticoagulants, protease inhibitors (indinavir, ritonavir, saquinavir), those individuals who had made a change within the last 6 weeks before randomisation and during the study in the medications that could interfere with the lipid profile (i.e., anti-hypertensive drugs, oral corticosteroids, thyroid hormones, retinoids, thiazidic derivative, hormone replacement therapy), those using drugs affecting lipid and glucose metabolism, weight reducing drugs and other drugs with known metabolic effects.

Individuals treated for/against obesity and had received medical treatment within the last 6 weeks (Orlistat, Sibutramine) and/or surgery (gastroplasty, bypass), and had body weight changes exceeding \( \pm 5\% \) of total body weight during the last three months before randomisation, were also excluded.

In addition, individuals were excluded if they had any of the following conditions: recent myocardial infarction (within 3 months prior to randomisation), current chronic pancreatitis, or identified risk or known history of acute pancreatitis, hepatic insufficiency, (AST and/or ALT > 2 times the upper normal limit (UNL)), acute alcohol intoxication, alcoholism, known cholelithiasis without cholecystectomy, renal failure or renal dysfunction (defined by serum creatinine levels > 135 µmol/L in males and > 110 µmol/L in females), known gastric or peptic ulcer or intestinal disease within the previous 3 months of randomisation.

Individuals with any other severe pathology such as cancer, mental illness, etc, which, in the opinion of the investigator, was found to possibly pose a risk to the individual or confound the results of the study, were all excluded.
Pregnant and lactating women were also excluded.

Furthermore, individuals having received an investigational drug in the last 30 days before date of randomisation, those who were unable or unwilling to comply with the protocol and those likely to withdraw from the study before its completion, were all excluded.

### 4.1.3 Screening

Two-hundred and fifty individuals responded to the advertisements and volunteered to take part in the study. Volunteers were sent a letter of invitation to take part in the study with information about the study (Appendix 2), and were later contacted by telephone for a screening interview (Appendix 3). Sixty-six individuals were invited to the clinic for a screening visit and their eligibility was assessed by evaluation of medical history, medical examination and intake of dairy products (with a self-administered food frequency questionnaire). Forty-four individuals were included and randomised (22 in the intervention/milk group; of which 8 were male and 14 female, 22 individuals were randomised to the control group; of which 8 were male and 14 female). Six participants were excluded before the study ended and a further two were excluded after the end of the study, i.e. thirty-six (36) participants fully completed the study.

### 4.1.4 Ethics

Written informed consent was obtained from all participants and it complied with the Declaration of Helsinki. The study protocol was approved by the Regional Committee for Medical Research Ethics (Region South), and is reported to Norwegian Social Science Data Services (Appendix 4). The study was also registered at www.clinicaltrials.gov.
4.1.5 Finances

The project is financed by funds from Norwegian Research Council, Opplysningskontoret for meieriprodukter (www.melk.no) and Tine BA.

4.2 Study design

This study was conducted between January 2005 and July 2007. It is a randomised parallel group intervention study, with 4 week baseline/ run-in period followed by a 6 months intervention period. Each participant entered the study at different dates; and the last participants were included in December 2006.

4.2.1 Randomisation

The subjects were assigned to the control or intervention groups by randomisation (participants were randomised in the order they came to the laboratory for tests on the day of randomisation; odd numbers were randomised to the intervention group and equal numbers were allocated to the control group). Due to the nature of the intervention, the study was not blinded.

4.2.2 Intervention

The participants in the milk group were instructed to increase their intake of dairy foods to at least three portions of dairy products each day (Appendix 5). They were not instructed to exclude or include any other foods/ food groups. The dairy products had to be chosen from the list below. The subjects in the control group were instructed to continue with their habitual diet.

<table>
<thead>
<tr>
<th>Milk (white or fermented):</th>
<th>Yoghurt/cottage cheese:</th>
<th>Cheese</th>
<th>Butter and butter spread:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 unit/portion = 100 –</td>
<td>1 portion= 40 grams or</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 portion= 10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Participants were instructed to maintain their usual diet in the run-in period. All participants were instructed to maintain their habitual physical activity throughout the study. They are also instructed to continue with possible drug prescriptions as normal.

**Dietary assessment and evaluation of compliance**

For accurate estimation of their baseline dietary intake, all participants completed a 3-day (2 week days and 1 weekend day) weighed dietary intake after the screening visit, prior to starting the intervention.
To check for compliance, the participants performed a further three 3-day weighed dietary intake at 2, 4 and 6 months respectively. The intermediate dietary records were used to reinforce the dietary advice, given for the milk group, and to strengthen compliance. The results of the last 3-day weighed dietary intake (at 6 months) were used when calculating dietary changes during the study.

The subjects were given detailed instruction on how to perform accurate weighed dietary intake records during the clinic visit (Appendix 6). Each subject was provided with a dietary record sheets and an electronic scale measuring with the precision of 2 g and a maximum of 2500 g. The use of household measures as substitutes for weighed amounts was accepted when it was impossible to weigh the food items. Liquid was measured in decilitres or grams. All subjects received oral and written instructions to continue their normal eating habits during the dietary registration. It was emphasized that the purpose was to record the usual food intake and that any temptations to change the diet in order to lose weight or simplify the recording should be avoided. They were asked to record the type and amount of all foods and drinks consumed. The subjects were instructed to record their dietary intake on three consecutive days, two weekdays and one weekend day. The subjects were provided with addressed pre-stamped envelopes for returning their completed food diaries; some however returned them electronically by e-mail. The dietary records were checked for completeness of description of foods and amounts. Subjects were asked about their habitual use of any multi- or single vitamin or mineral supplements, however, these data were not included in the dietary analysis. Daily intakes of energy and nutrients were computed using a food database and software system developed at the Institute for Nutrition Research, University of Oslo (Beregn).

Details of dietary intervention
Participants in the milk group had to choose at least 3 portions per day from a list of dairy products provided. They had to include at least 1 portion from the cheese or butter group daily. The other two or more portions of dairy products could be chosen from any of the other groups; the milk group and yoghurt group, or they could choose...
to have all portions from cheese or butter. Additionally, participants were free to choose dairy products from a “luxury group”, but not on a daily basis due to its high calorie content (see list below).

All participants were provided with a diary in which they were informed to record their daily intake of dairy products (Appendix 7). This diary was returned at the next clinic visit and used to check for compliance in both the milk group and the control group.

The participants purchased the dairy products of their choosing from their local supermarket. They were encouraged to collect the shopping receipts and were reimbursed the amount at the end of the study.

Luxury group

- Ice creams (milk based)
- Sweetened milk drinks (milkshake, cocoa etc.)
- Dairy desserts (rice pudding, vanilla sauce, milk puddings etc.)
- Cream, crème fraiche and sour cream
- Sour cream porridge
- Cheeses with high fat content > 30% (brie, camembert etc.)

4.2.3 Clinical investigations

Each subject met for five visits; a screening visit, randomisation visit (baseline), a visit at 2 and 4 months and a final visit at 6 months (Appendix 8). Clinical and laboratory tests were conducted at randomisation/baseline and at the end of the
intervention at 6 months; some parameters were also tested during the intervention period at 2 and 4 months (Appendix 8).

**Anthropometry and body composition**

Body weight was measured to the nearest 0.1 kg with a calibrated digital scale while the subjects were dressed in light clothes and no shoes. Waist circumference was with the subject in the standing position, with measurements obtained midway between the lowest rib and the iliac crest at the end of gentle expiration. The average of two readings was recorded. Height was measured with a mobile wall-mounted Stadiometer to the nearest cm. BMI was calculated via standard equation (kg/m²).

Body composition was measured by dual-energy X-ray absorptiometry (DEXA) at baseline and repeated at 6 months (DPX-L; Lunar Co, Madison, WI). CVs for body fat mass and fat-free mass were <2%. The DEXA system provides whole-body and regional estimates of three main components: bone mineral, bone-free fat free mass (FFM), and fat mass. For the purpose of this study, fat mass was of particular interest. The DEXA scan provided estimates of fat mass as percentage total fat mass, total body fat mass in kg and regional estimations such as fat mass for both trunk fat and abdominal fat mass.

**Blood pressure**

Blood pressure was measured with the subject seated in an upright position in a chair, after 5–10 minutes rest, with the arm supported at heart level. Blood pressure was measured with an appropriately sized cuff using a standard, calibrated mercury sphygmomanometer. Three readings were taken and the average value recorded. The same arm was used for every blood pressure measurement at each visit.

**Blood samples**

Blood samples were drawn from an antecubital vein in the morning after an overnight fast (≥10 hours). Routine laboratory methods were used to measure serum total cholesterol, HDL and LDL cholesterol, serum triglycerides, apolipoprotein (apo) A-I
and apo B, plasma glucose, HbA1c, insulin, and C-peptide. The CVs for these methods were <7%. The fatty acid composition of serum cholesteryl esters was evaluated by gas chromatography of fatty methyl esters (GLC 5890; Hewlett-Packard, Palo Alto, CA) after separation of the serum lipids by thin-layer chromatography, as described previously (26). Concentrations of high-sensitivity C-reactive protein and complement factors (C3 and C4) were measured with an automated Konelab Analyzer (Thermo Fisher Scientific, Vantaa, Finland). Intra- and interassay CVs for all 3 methods were <5%. Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured with an automated immunoassay system (Immulite 1000; Siemens, Los Angeles, CA). The CVs for these methods were <7%. The serum 25-hydroxyvitamin D concentration was analyzed by using enzyme immunoassay kits (IDS, Boldon, United Kingdom). Reproducibility was ensured by adhering to the Vitamin D External Quality Assessment Scheme. Standardized concentrations of serum 25-hydroxyvitamin D were used in the analysis. Leptin concentrations were measured by using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). All samples were analyzed in duplicate. The interassay and intraassay CVs were 5.3% and 4.6% for serum 25-hydroxyvitamin D and 3.2% and 5.4% for leptin, respectively. Isoprostane excretion in urine was determined as described previously (27). Vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and adiponectin were analyzed in serum with commercial enzyme-linked immunosorbent assay methods (R&D Systems, Abingdon, United Kingdom). Citrated plasma was used for von Willebrand Factor (vWF) measurement by using an enzyme-linked immunosorbent assay method (Asserachrom Stago Diagnostica, Asnieres, France), and plasminogen activator inhibitor-1 (PAI-1) activity was measured by Spectrolyse PL (Biopool AB, Umeå, Sweden). The interassay CVs were 5.2% for VCAM-1, 5.3% for E-selectin, 9.5% for adiponectin, 8.0% for vWF, and 4.4% for PAI-1. The analyses concerning glucose metabolism and lipid metabolism were performed locally at the routine laboratory at the Oslo University Hospital, Rikshospitalet. Measurements of adiponectin, leptin, 25-hydroxyvitamin D, fatty acid composition, and markers of inflammation,
endothelial function, and oxidative stress were analyzed at collaborating centres in Sweden (Uppsala) and Finland (Helsinki).

### 4.2.4 Sample size and statistical analysis

In order to detect a clinically relevant difference of ≈1.5 cm in waist circumference between the intervention and control groups at a significance level of $P < 0.05$ and a power of 80% a sample size of 120 participants was necessary for the overall Nordic study as a whole. The Norwegian study population consisted of 36 participants and the data presented include all the participants who completed the study.

Parametric tests were used for normally distributed data. Student’s t test was used to test compare the difference in change from baseline to 6 months between the milk and control groups (Independent-samples t test). Student’s t test was also used to evaluate the differences in change between baseline and 6 months within the groups (One-sample t test and Paired-samples t test for deltaxvalue). We also tested for difference between baseline values between the two groups. For data that were not normally distributed Wilcoxon test was used. Statistical package for the Social Sciences (SPSS) version 17.0 and 18.0 for Windows was used for the statistical analysis.
5. Results

5.1 Subjects

More than 250 subjects were initially screened either by telephone interview or an initial clinic visit (exact number is unknown due to incomplete recording of all telephone calls). Sixty-six individuals were invited to a screening visit and, 44 subjects were included and randomized into the study (16 male and 28 female subjects). Only 36 of these subjects completed the study, 17 and 19 randomized into the milk and control groups respectively. The reasons for excluding the eight remaining subjects were all related to not complying with the study protocol. Three subjects started hypertensive treatment, and another commenced pharmaceutical weight reducing therapy. One subject was diagnosed with hypothyroidism, another subject was diagnosed with a large abdominal cyst, both of which were considered to interfere with the results of the study. Two subjects withdrew the consent from the study (one was lost to follow-up and one was unwilling to comply with the study protocol. Of the excluded subjects, 5 subjects belonged to the intervention group (1 male, 4 female) and 3 (1 male, 2 female) to the control group.

5.2 Baseline characteristics

5.2.1 Physical characteristics

The control and milk groups were comparable with regards to age and sex distribution at the start of the study. Baseline characteristics were mainly similar (Table 1), but some differences between men and women within the milk and control groups for some of the variables were observed. The women and men differed significantly in weight and body composition. Women in the milk group were significantly heavier than men (95.7 kg ± 6.1 and 80.4 ± 8.8 respectively, P =0.001). The women in the
milk group also had significantly higher percentage body fat than men (43.6% ± 6.3 versus 30.7% ± 4.6, P = 0.000) at baseline. In the control group the body weight was similar for men and women at baseline, however, the women had significantly higher body fat mass than men (35.8 kg ± 6.4 versus 26.7 kg ± 6.2, P = 0.014), and the women also had higher percentage body fat (43.3% ± 5.7 versus 29.7% ± 3.6, P = 0.000).

Table 1: Baseline characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Milk</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/ male (n)</td>
<td>12/7</td>
<td>10/7</td>
<td>0.80</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55 ± 7.4</td>
<td>54 ± 6.9</td>
<td>0.71</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.5 ± 0.94</td>
<td>5.9 ± 1.4</td>
<td>0.32</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.6 ± 0.97</td>
<td>3.9 ± 1.0</td>
<td>0.37</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.3 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>0.48</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.8 ± 1.3</td>
<td>2.0 ± 1.8</td>
<td>0.81</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88.6 ± 1.0</td>
<td>86.7 ± 10.8</td>
<td>0.59</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>103.0 ± 7.9</td>
<td>103.5 ± 7.8</td>
<td>0.83</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.6 ± 3.1</td>
<td>29.4 ± 2.7</td>
<td>0.83</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>133 ± 14</td>
<td>130 ± 11</td>
<td>0.38</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>85 ± 7</td>
<td>85 ± 4</td>
<td>0.99</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.4 ± 0.5</td>
<td>5.5 ± 0.6</td>
<td>0.47</td>
</tr>
<tr>
<td>No. of Metabolic syndrome criteria fulfilled</td>
<td>2.8 ± 0.8</td>
<td>2.5 ± 0.7</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*All values are given as means in grams ± SD. The data was analysed by t-tests. *Significant value (<0.05).

5.2.2 Metabolic variables

The metabolic variables were also matched for the control and milk group, with only some difference between sexes within the two groups. Adiponectin levels at baseline were higher among women in both control and milk groups (10.0 mg/l ± 6.3 versus 5.3mg/l ± 2.4, P =0.034 for women and men in the control group, 13.1mg/l ± 6.2 versus 5.4mg/l ± 2.4, P = 0.004 for women and men in the milk group). There was also a difference in the inflammatory markers C3 and C4 between men and women in the control group at baseline, women had significantly higher values.

5.2.3 Energy and nutrient intake

With regards to dietary intake, energy and nutrient intakes; there were no significant difference between the control and milk group at baseline. However, men in both groups had a higher mean energy intake at baseline, significantly so only within the milk group, but there were no difference between the two groups.
The control group had a significantly higher percentage energy intake from protein at baseline (18 E% vs. 16 E%, $P = 0.042$), and a significantly lower vitamin D intake (6.4 µg/day vs. 10.2 µg/day in the milk group, $P = 0.034$).

There were some differences in the macronutrient intake between men and women within the milk group. The men had a significantly higher daily protein intake, total fat intake and a tendency for a higher intake of the different fatty acids, including saturated fat, MUFA and PUFA than women in the same group. The men in the control group similarly had a higher protein intake, an only a tendency for a higher intake of PUFA compared to women in the same group.

The daily fat intake and the proportions of different fatty acids in the diet did not differ significantly for the two groups at baseline. There was significant difference between the sexes at baseline within the groups for; women in the control group had a significantly higher intake of palmitoleic acid (16:1) ($3.44 \pm 0.90$ and $2.29 \pm 1.36$ for women and males, respectively, $P = 0.04$). The daily intake of milk fat (sum of fat from butter and butter containing spreads, milk, yoghurt, cottage cheese, cheese, crème and ice-cream) was about 11 g in both groups.

**5.2.4 General health of the subjects/ Degree of Metabolic Syndrome**

All subjects had to fulfil $\geq 2$ of the criteria for the metabolic syndrome as defined by the NCEP. The criteria most commonly fulfilled were waist circumference (97%; 35 of 36 subjects) and hypertension (present in 83.3% of the subjects, 30 subjects in total, 14 in milk and 16 in the control group, respectively). The average number of criteria fulfilled at baseline was $2.8 (\pm 0.8)$ in the control group and $2.5 (\pm 0.7)$ in the milk group.

At the end of the study period the figures were $2.7 (\pm 0.8)$ and $2.7 (\pm 1.1)$, in the control and milk groups respectively, which was not shown to be a statistically significant change neither within nor between the groups.
5.3 Changes during the intervention period

5.3.1 Changes in dairy food intake

The milk group significantly increased their intake of all dairy products during the study period, compared to the control group (Table 2). Cheese intake increased on average by 18 g/d in the milk group (P = 0.009), i.e. by half a portion of cheese daily. Butter intake also increased in the milk group (P = 0.036), but fell short of reaching significance level compared to the control group (P = 0.057).

The intake of fluid milk and yoghurt increased by an average of 163 g/d (P = 0.005) in the milk group. There was a tendency for women in the milk group to have the largest increase in their intake of total milk products (fluid milk and yoghurt) (P = 0.061).

The control group reduced the daily intake of dairy products by nearly half a portion to about one and a half portion daily (of total milk, yoghurt, cheese and butter), from approximately 2 portions at baseline. The milk group had a similar intake at baseline, with an intake of dairy of approximately 2 portions per day. This increased to a mean intake of approximately 3 portions daily.
Table 2. Dietary intake in the control group and the milk group at baseline and 6 months, as calculated from 3-day food records

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Control (n=16-18)</th>
<th>Milk (n=14-17)</th>
<th>Δ6-0</th>
<th>P-value</th>
<th>Control vs. Milk</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mo</td>
<td>6 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total milk</td>
<td>144 ± 119</td>
<td>131 ± 101</td>
<td>-13 ± 78</td>
<td>0.50</td>
<td>170 ± 169</td>
<td>333 ± 134</td>
</tr>
<tr>
<td>Fluid milk</td>
<td>72 ± 91</td>
<td>80 ± 99</td>
<td>9 ± 69</td>
<td>0.60</td>
<td>145 ± 152</td>
<td>220 ± 89</td>
</tr>
<tr>
<td>Yoghurt etc</td>
<td>31 ± 53</td>
<td>17 ± 43</td>
<td>-15 ± 55</td>
<td>0.28</td>
<td>11 ± 32</td>
<td>35 ± 56</td>
</tr>
<tr>
<td>Cheese</td>
<td>20 ± 26</td>
<td>15 ± 14</td>
<td>-5 ± 26</td>
<td>0.44</td>
<td>19 ± 21</td>
<td>36 ± 23</td>
</tr>
<tr>
<td>Butter</td>
<td>6 ± 9</td>
<td>5 ± 8</td>
<td>-1 ± 10</td>
<td>0.80</td>
<td>7 ± 6</td>
<td>12 ± 10</td>
</tr>
<tr>
<td>Other milk</td>
<td>17 ± 24</td>
<td>14 ± 23</td>
<td>-3 ± 34</td>
<td>0.76</td>
<td>7 ± 14</td>
<td>33 ± 46</td>
</tr>
<tr>
<td>Fish</td>
<td>81 ± 54</td>
<td>84 ± 67</td>
<td>3 ± 97</td>
<td>0.90</td>
<td>61 ± 48</td>
<td>72 ± 47</td>
</tr>
<tr>
<td>Red meat</td>
<td>132 ± 127</td>
<td>101 ± 87</td>
<td>-31 ± 128</td>
<td>0.32</td>
<td>123 ± 60</td>
<td>74 ± 60</td>
</tr>
<tr>
<td>Poultry</td>
<td>35 ± 48</td>
<td>37 ± 43</td>
<td>2 ± 47</td>
<td>0.86</td>
<td>22 ± 35</td>
<td>59 ± 106</td>
</tr>
<tr>
<td>White bread</td>
<td>63 ± 63</td>
<td>92 ± 71</td>
<td>28 ± 80</td>
<td>0.15</td>
<td>61 ± 59</td>
<td>80 ± 45</td>
</tr>
<tr>
<td>Wholegrain bread</td>
<td>72 ± 52</td>
<td>56 ± 79</td>
<td>-16 ± 109</td>
<td>0.55</td>
<td>52 ± 50</td>
<td>64 ± 99</td>
</tr>
<tr>
<td>Cereals</td>
<td>25 ± 36</td>
<td>32 ± 39</td>
<td>7 ± 45</td>
<td>0.54</td>
<td>51 ± 53</td>
<td>33 ± 38</td>
</tr>
<tr>
<td>Fruits</td>
<td>173 ± 193</td>
<td>138 ± 168</td>
<td>-35 ± 94</td>
<td>0.13</td>
<td>179 ± 130</td>
<td>147 ± 121</td>
</tr>
<tr>
<td>Vegetables</td>
<td>188 ± 123</td>
<td>146 ± 100</td>
<td>-42 ± 122</td>
<td>0.16</td>
<td>152 ± 72</td>
<td>100 ± 62</td>
</tr>
<tr>
<td>Potatoes</td>
<td>69 ± 42</td>
<td>51 ± 59</td>
<td>-18 ± 70</td>
<td>0.29</td>
<td>81 ± 58</td>
<td>59 ± 70</td>
</tr>
<tr>
<td>Alcohol beverages</td>
<td>163 ± 203</td>
<td>278 ± 407</td>
<td>114 ± 326</td>
<td>0.16</td>
<td>143 ± 240</td>
<td>161 ± 277</td>
</tr>
<tr>
<td>Sugar &amp; sweets</td>
<td>26 ± 74</td>
<td>38 ± 73</td>
<td>12 ± 75</td>
<td>0.51</td>
<td>21 ± 32</td>
<td>12 ± 15</td>
</tr>
<tr>
<td>Cakes, buns, cookies</td>
<td>33 ± 34</td>
<td>31 ± 36</td>
<td>-2 ± 35</td>
<td>0.86</td>
<td>49 ± 53</td>
<td>44 ± 73</td>
</tr>
<tr>
<td>Soft drinks</td>
<td>76 ± 217</td>
<td>76 ± 133</td>
<td>0 ± 357</td>
<td>1.00</td>
<td>36 ± 80</td>
<td>65 ± 311</td>
</tr>
<tr>
<td>Fruit juices</td>
<td>155 ± 260</td>
<td>132 ± 195</td>
<td>-24 ± 97</td>
<td>0.34</td>
<td>68 ± 87</td>
<td>48 ± 74</td>
</tr>
</tbody>
</table>

1 All values are given as means in grams ± SD. 0 mo = baseline data, 6 mo = end of the study. Δ6-0 = difference between 6 months and baseline data.
2 The data was analysed by t-tests.
3 a p-value for change between baseline and 6 months within group.
4 b p-value between groups. Difference between change in control group versus change in milk group.
5 * Significant value (<0.05).

5.3.2 Changes in overall food choices

With regards to the other food groups there were no significant differences in intake between the two groups after six months (Table 2). There was no tendency for a reduction in fish intake as was the case for the Nordic population as a whole. Fish intake increased slightly in both groups in the Norwegian population, but not statistically significant. Accompanying the reduction seen in fish intake in the Nordic population, there was an increase in poultry intake; this was not, however, seen in the Norwegian study subjects. Interestingly, in this Norwegian study population a significant reduction in the intake of red meat was observed in the milk group (P = 0.007). The milk group also reduced the vegetable intake after the six months intervention (P = 0.003), but not significantly compared to the control group. When analyzed combined, fruit and vegetable intake tended to decrease, but not statistically significant, in both the control (P = 0.054) and milk groups (P = 0.081).

Otherwise, there were no clear differences between the two groups after the intervention period.
Some differences within the sexes were seen, e.g. with regards to fruit and vegetable intake combined. There was a statistically significant decrease in fruit and vegetable intake among the women in the control group (P = 0.043). Men in the control group also significantly reduced cereal intake, while the women increased their intake (P = 0.042).

5.3.3 Changes in energy and nutrient intake

The energy intake did not differ significantly between the groups during the study (Table 3), and in contrast to the Nordic study population as a whole, no tendency for an increase in the mean energy intake in the milk group in comparison to the control group was observed in the Norwegian subjects.

There was no statistically significant differences between the milk and control groups with regards to change in macronutrient intake, only a tendency for an increase in percentage energy from saturated fatty acids (P = 0.056) and also a non-significant decrease in trans fatty acids (P = 0.082) in the milk group. In comparison, in the Nordic study population, the increased intake of dairy products in the milk group was associated with a higher intake of protein, fat, and especially saturated fat.

Furthermore, in this Norwegian study population, the percentage energy intake from PUFA was significantly decreased after six months (P = 0.028) and percentage energy intake from MUFA tended to decrease (P = 0.09). The control group on the other hand, showed a significant reduction in total fat (P = 0.020), a tendency for decreased saturated fat intake (P = 0.074), and also a significant reduction in the intake of both MUFA and PUFA (P = 0.040 and 0.013, respectively). However, there were no significant differences between the control and milk groups.

The intervention did not influence protein intake, total carbohydrate intake or sugar intake, or the intake of alcohol in the Norwegian study population, which is in contrast to the Swedish study population. The intervention period also led to increased
intake of dietary cholesterol and calcium in the milk group compared to the control group (P = 0.044 and 0.007 respectively) (Table 3).

**Table 3. Intake of energy and nutrients in the control group and the milk group at baseline and 6 months^1**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=18)</th>
<th>Milk (n=16-18)</th>
<th>Control vs. Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mo</td>
<td>6 mo</td>
<td>ð 6-0 P-value</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>8312 ± 2711</td>
<td>7543 ± 2345</td>
<td>-769 ± 1967 0.14</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>88 ± 34</td>
<td>80 ± 23</td>
<td>-7.8 ± 27.1 0.24</td>
</tr>
<tr>
<td>(E%)</td>
<td>18.0 ± 3.1</td>
<td>18.6 ± 4.1</td>
<td>0.64 ± 4.3 0.53</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>196 ± 58</td>
<td>184 ± 58</td>
<td>-11.5 ± 83.9 0.46</td>
</tr>
<tr>
<td>(E%)</td>
<td>40.1 ± 9.3</td>
<td>42.1 ± 7.6</td>
<td>2.9 ± 7.7 0.28</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>84 ± 59</td>
<td>72 ± 37</td>
<td>-12.5 ± 36.3 0.16</td>
</tr>
<tr>
<td>Fat, total (g)</td>
<td>81 ± 36</td>
<td>64 ± 27</td>
<td>-17.0 ± 28.2 0.020*</td>
</tr>
<tr>
<td>(E%)</td>
<td>35.6 ± 7.9</td>
<td>30.5 ± 6.1</td>
<td>-5.1 ± 9.0 0.029*</td>
</tr>
<tr>
<td>SAT (g)</td>
<td>27.6 ± 45.4</td>
<td>23.4 ± 9.1</td>
<td>-4.2 ± 9.3 0.074</td>
</tr>
<tr>
<td>(E%)</td>
<td>12.3 ± 3.6</td>
<td>11.4 ± 2.6</td>
<td>-0.9 ± 3.6 0.28</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>28.3 ± 14.0</td>
<td>21.5 ± 10.9</td>
<td>-6.9 ± 13.1 0.040*</td>
</tr>
<tr>
<td>(E%)</td>
<td>12.4 ± 3.2</td>
<td>10.2 ± 3.4</td>
<td>-2.2 ± 4.6 0.063</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>16.9 ± 9.0</td>
<td>12.8 ± 6.2</td>
<td>-4.1 ± 6.3 0.013*</td>
</tr>
<tr>
<td>(E%)</td>
<td>7.3 ± 2.5</td>
<td>6.1 ± 1.9</td>
<td>-1.3 ± 2.1 0.024*</td>
</tr>
<tr>
<td>Trans (g)</td>
<td>0.7 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>-0.1 ± 0.4 0.24</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>11.5 ± 14.4</td>
<td>19.4 ± 24.1</td>
<td>7.9 ± 23.2 0.17</td>
</tr>
<tr>
<td>(E%)</td>
<td>4.2 ± 5.9</td>
<td>6.5 ± 7.4</td>
<td>2.4 ± 7.5 0.20</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>394 ± 196</td>
<td>315 ± 153</td>
<td>-79 ± 189 0.095</td>
</tr>
<tr>
<td>(µg)</td>
<td>6.4 ± 2.8</td>
<td>5.4 ± 3.9</td>
<td>-1.0 ± 4.8 0.39</td>
</tr>
</tbody>
</table>

^1 All values are given as means in grams ± SD. 0 mo = baseline data, 6 mo = end of the study. ð 6-0 = difference between 6 months and baseline data.

The data was analysed by t-tests.

a p-value for change between baseline and 6 months within group

b p-value between groups. Difference between change in control group versus change in milk group.

* Significant value (<0.05).

5.3.4 Changes in milk fat intake

The daily intake of milk fat (sum of fat from butter and butter containing spreads, milk, yoghurt, cottage cheese, cheese, cream and ice cream) was about 11 g in both groups at baseline. The intake increased in the milk group during the intervention, and the changes of milk fat intake in the two groups were highly significantly different (P = 0.000). The actual milk fat intake in the milk group increased to about 25 grams per day.

5.3.5 Fatty acid composition of serum cholesteryl esters

The fatty acid composition of the serum cholesteryl esters, reflecting the fatty acid composition of the diet, was similar during the two test periods (Table 4). The only exception was a highly significant increase of the proportion of pentadecanoic acid (15:0, P = 0.006) seen in the Milk group, and also a significant difference in myristic acid (14:0, P = 0.015), as it increased in the milk group. 15:0 is a specific marker for
intake of dairy fat and the data confirms that these subjects had significantly increased their intake of fat from milk products.

In spite of the increase of dairy products and milk fat in the diet, there were no other changes in the proportions of other saturated or monounsaturated fatty acids in serum and the proportions of polyunsaturated long-chain fatty acids of the n-6 and n-3 family remained unchanged. These data are in accordance with the nutrient data, indicating a virtually unchanged dietary fat composition as concerns the major fatty acids.

Table 4. Fatty acid composition of serum cholesteryl esters in the control group and the milk group at baseline and 6 months

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control (n=18)</th>
<th>Milk (n=17)</th>
<th>Control vs. Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mo</td>
<td>6 mo</td>
<td>δ 6-0</td>
</tr>
<tr>
<td>% of fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristic, 14:0</td>
<td>0.82±0.19</td>
<td>0.75±0.20</td>
<td>-0.07±0.12</td>
</tr>
<tr>
<td>Palmitic, 16:0</td>
<td>11.29±0.61</td>
<td>11.25±0.61</td>
<td>-0.03±0.58</td>
</tr>
<tr>
<td>Palmitoleic, 16:1</td>
<td>2.93±1.17</td>
<td>2.86±1.13</td>
<td>-0.07±0.40</td>
</tr>
<tr>
<td>Stearic, 18:0</td>
<td>0.89±0.23</td>
<td>0.82±0.17</td>
<td>-0.07±0.11</td>
</tr>
<tr>
<td>Oleic, 18:1</td>
<td>51.47±3.62</td>
<td>52.04±3.88</td>
<td>0.57±2.68</td>
</tr>
<tr>
<td>Linoleic, 18:2n-6</td>
<td>0.89±0.45</td>
<td>0.89±0.45</td>
<td>0.00±0.33</td>
</tr>
<tr>
<td>α-Linolenic, 18:3n-3</td>
<td>0.60±0.14</td>
<td>0.57±0.15</td>
<td>-0.03±0.14</td>
</tr>
<tr>
<td>Dihomo-γ-Linolenic, 20:3n-6</td>
<td>0.78±0.18</td>
<td>0.76±0.17</td>
<td>-0.02±0.08</td>
</tr>
<tr>
<td>Arachidonic, 20:4n-6</td>
<td>6.71±0.94</td>
<td>6.82±1.19</td>
<td>0.11±0.60</td>
</tr>
<tr>
<td>Eicosapentaenoic, 20:5n-3</td>
<td>3.27±1.32</td>
<td>2.66±1.48</td>
<td>-0.60±1.03</td>
</tr>
<tr>
<td>Docosahexaenoic, 22:6n-3</td>
<td>1.16±0.25</td>
<td>1.08±0.22</td>
<td>-0.15±0.36</td>
</tr>
</tbody>
</table>

All values are given as means in grams ± SD. 0 mo = baseline data, 6 mo = end of the study. δ 6-0 = difference between 6 months and baseline data.

The data was analyzed by t-tests.

*Significant value (<0.05).

5.3.6 Changes to anthropometric and clinical variables

The Norwegian population remained unchanged with regards to body weight, BMI and waist circumference during the intervention (Table 5), as did body fat mass and the proportion of body fat. There was no significant change in blood pressure between the two groups, only a slight tendency for an increase in systolic blood pressure in the milk group (P = 0.064). Similar changes were found in the Nordic population study as a whole.
The changes in plasma glucose, insulin, C-peptide, or HbA1c were not significantly different between the milk and the control groups (Table 6). There was, however, a tendency to a difference in fasting insulin concentrations ($P = 0.078$) and a significant difference between changes in the homeostasis model of assessment (HOMA) index ($P = 0.037$) between the groups. The HOMA values are based on the Nordic study population as a whole, as the Norwegian data was not made available for separate analysis.

There was no significant change in the lipid profile of the Norwegian subjects (Table 7).
Table 7. Lipid variables in control group and the milk group at baseline and 6 months.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 mo</th>
<th>6 mo</th>
<th>δ 6-0</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>0 mo</th>
<th>6 mo</th>
<th>δ 6-0</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control vs. Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.5 ± 0.95</td>
<td>5.5 ± 0.94</td>
<td>0.00 ± 0.40</td>
<td>1.0</td>
<td>5.92 ± 1.41</td>
<td>5.94 ± 1.46</td>
<td>0.02 ± 0.72</td>
<td>0.92</td>
<td>0.93</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.6 ± 0.97</td>
<td>3.7 ± 0.77</td>
<td>0.11 ± 0.71</td>
<td>0.52</td>
<td>3.91 ± 1.02</td>
<td>4.08 ± 1.02</td>
<td>0.17 ± 0.81</td>
<td>0.40</td>
<td>0.80</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.28 ± 0.35</td>
<td>1.26 ± 0.35</td>
<td>-0.02 ± 0.18</td>
<td>0.61</td>
<td>1.39 ± 0.40</td>
<td>1.34 ± 0.40</td>
<td>-0.05 ± 0.20</td>
<td>0.32</td>
<td>0.67</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.84 ± 1.33</td>
<td>1.84 ± 1.29</td>
<td>-0.005 ± 0.69</td>
<td>0.97</td>
<td>2.07 ± 1.76</td>
<td>2.21 ± 1.92</td>
<td>0.13 ± 0.70</td>
<td>0.42</td>
<td>0.35</td>
</tr>
<tr>
<td>B (g/l)</td>
<td>1.08 ± 0.27</td>
<td>1.05 ± 0.25</td>
<td>-0.03 ± 0.09</td>
<td>0.14</td>
<td>1.16 ± 0.33</td>
<td>1.11 ± 0.34</td>
<td>-0.04 ± 0.17</td>
<td>0.28</td>
<td>0.78</td>
</tr>
<tr>
<td>A-I (g/l)</td>
<td>1.37 ± 0.27</td>
<td>1.40 ± 0.29</td>
<td>0.03 ± 0.13</td>
<td>0.40</td>
<td>1.39 ± 0.25</td>
<td>1.42 ± 0.20</td>
<td>0.03 ± 0.16</td>
<td>0.47</td>
<td>0.98</td>
</tr>
</tbody>
</table>

<sup>a</sup> All values are given as means in grams ± SD. 0 mo = baseline data, 6 mo = end of the study. δ 6-0 = difference between 6 months and baseline data. The data was analysed by t-tests. P-value for change between baseline and 6 months within group, p-value between groups. Difference between change in control group versus change in milk group.

One significant observation was seen in the change in E-selectin, a marker of endothelial function in the milk group. This marker significantly decreased in the Norwegian milk group compared to the control group. This is in contrast to the Nordic study population as a whole.

Otherwise, no significant differences were observed between the groups with regards to the variables related to inflammatory response (leptin, IL-6, hs-CRP, TNF-α, C3, and C4), endothelial function (vWF), fibrinolysis (PAI-1) and oxidative stress (Table 8). The serum concentration of 25-hydroxyvitamin D also remained unchanged.

Table 8. Markers of inflammation, endothelial function and oxidative in control group and the milk group at baseline and 6 months.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 mo</th>
<th>6 mo</th>
<th>δ 6-0</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>0 mo</th>
<th>6 mo</th>
<th>δ 6-0</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control vs. Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (mg/l)</td>
<td>8.1 ± 5.7</td>
<td>8.01 ± 5.5</td>
<td>-0.1 ± 3.0</td>
<td>0.94</td>
<td>9.9 ± 6.3</td>
<td>10.4 ± 6.4</td>
<td>0.5 ± 2.1</td>
<td>0.37</td>
<td>0.55</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>27874 ± 20649</td>
<td>24498 ± 1251</td>
<td>-3376 ± 9290</td>
<td>0.13</td>
<td>29075 ± 19819</td>
<td>30634 ± 18438</td>
<td>1559 ± 12962</td>
<td>0.61</td>
<td>0.19</td>
</tr>
<tr>
<td>hsCRP (mg/l)</td>
<td>2.84 ± 2.83</td>
<td>2.59 ± 2.83</td>
<td>-0.25 ± 1.83</td>
<td>0.56</td>
<td>4.04 ± 3.21</td>
<td>4.57 ± 3.68</td>
<td>0.53 ± 1.65</td>
<td>0.20</td>
<td>0.19</td>
</tr>
<tr>
<td>C3 (g/l)</td>
<td>1.30 ± 0.25</td>
<td>1.29 ± 0.25</td>
<td>-0.004 ± 0.15</td>
<td>0.90</td>
<td>1.40 ± 0.20</td>
<td>1.42 ± 0.20</td>
<td>0.02 ± 0.14</td>
<td>0.49</td>
<td>0.57</td>
</tr>
<tr>
<td>C4 (g/l)</td>
<td>0.30 ± 0.8</td>
<td>0.31 ± 0.09</td>
<td>0.004 ± 0.05</td>
<td>0.46</td>
<td>0.31 ± 0.82</td>
<td>0.32 ± 0.08</td>
<td>0.004 ± 0.04</td>
<td>0.64</td>
<td>0.99</td>
</tr>
<tr>
<td>VCAM (ng/ml)</td>
<td>607 ± 170</td>
<td>580 ± 135</td>
<td>-27 ± 65</td>
<td>0.09</td>
<td>565 ± 109</td>
<td>522 ± 134</td>
<td>-42 ± 78</td>
<td>0.039</td>
<td>0.52</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>33.1 ± 12.7</td>
<td>34.0 ± 8.1</td>
<td>0.8 ± 7.5</td>
<td>0.64</td>
<td>40.4 ± 15.6</td>
<td>32.2 ± 9.88</td>
<td>-8.2 ± 11.1</td>
<td>0.013*</td>
<td>0.008*</td>
</tr>
<tr>
<td>U (U/ml)</td>
<td>20.4 ± 8.8</td>
<td>22.0 ± 11.4</td>
<td>1.6 ± 40.5</td>
<td>0.50</td>
<td>21.1 ± 8.4</td>
<td>18.6 ± 7.6</td>
<td>-2.5 ± 7.2</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)</td>
<td>83.0 ± 29.45</td>
<td>89.36 ± 29.47</td>
<td>6.34 ± 30.55</td>
<td>0.38</td>
<td>99.38 ± 42.05</td>
<td>108.39 ± 29.40</td>
<td>9.01 ± 32.02</td>
<td>0.24</td>
<td>0.79</td>
</tr>
</tbody>
</table>

<sup>a</sup> All values are given as means in grams ± SD. 0 mo = baseline data, 6 mo = end of the study. δ 6-0 = difference between 6 months and baseline data. The data was analysed by t-tests. P-value for change between baseline and 6 months within group, p-value between groups. Difference between change in control group versus change in milk group. Significant value (<0.05).
Regression analyses relating the changes in the proportions of 15:0 in serum cholesteryl esters (specifically mirroring the changes in intake of milk fat) with those of the clinical variables showed that the increase in serum cholesterol was significantly related to that of 15:0 ($r = 0.24$, $P = 0.011$), which suggests that the increased cholesterol concentrations were in fact due to the increased intake of milk fat. There was no significant correlation between changes in the HOMA index and 15:0 in the serum cholesteryl esters.

### 5.3.7 Effects related to habitual calcium intake

As explained in the published scientific paper, there may be a possible threshold for the effect of dairy products and dietary calcium on blood pressure. This threshold has been suggested at a calcium intake of 700–800 mg/d (108). Among the Norwegian study population about 40 of the subjects in the milk group had a calcium intake at baseline that exceeded this limit (7 out of 17 subjects). In a post hoc analysis, the participants were thus divided into 2 groups with a daily calcium intake below ($n = 25$) and above ($n = 10$) 700 mg, respectively, and the effects on body composition (BMI, waist circumference, SAD, body fat mass, and body fat proportion) and blood pressure were tested.

Among the participants with a low calcium intake, there was a significant treatment effect for waist circumference ($P = 0.023$). In the subjects with a low calcium intake at baseline, waist circumference was $105.1 \pm 8.6$ cm at baseline and $101.5 \pm 9.7$ cm at 6 months in the milk group, but remained essentially unchanged in the control group ($103.3 \pm 7.5$ and $101.7 \pm 7.3$ cm, respectively). There were no other effects related to the level of calcium intake on BMI, body fat mass, or the proportion of body fat.

Similar results were observed in the Nordic study population as a whole with regards to treatment effect on waist circumference, in addition, the milk group in Nordic study population had a significant treatment effect for diastolic but not for systolic blood pressure in the Nordic study population.
6. Discussion

6.1 Biomarker of milk fat intake

In this controlled 6-months dietary intervention study, dietary records and analyses of a serum biomarker for intake of milk fat (pentadecanoic acid, 15:0) were used to verify that the participants in the milk group had significantly increased their intake of dairy products (Table 2). The participants received no advice concerning dietary changes, except for the instruction to increase the intake of dairy products. On the contrary, the intention was to allow for adjustments of energy intake and other compensatory dietary changes to study the consequences of a higher intake of dairy foods.

6.2 Changes in dairy intake

The major change in dairy intake in the milk group was an increase in milk intake, ordinary as well as fermented, and of cheese. The subjects mainly were instructed to consumed low-fat dairy or moderate-fat dairy products, which probably explain the rather small non-significant mean increase in saturated fat. The Swedish participants, on the other hand, included more full-fat products, as is seen in a resultant increased saturated fat intake for the Nordic study population as a whole.

6.3 Changes in energy intake

The mean energy intake did not change significantly either during the intervention period. There was, in fact, a non-significant decrease in both the milk group and the control group. This indicates that the higher intake of dairy products in the milk group was balanced by a reduced intake of energy and nutrients, including saturated fatty acids, from other food groups.
In comparison, in the Nordic study population as a whole, there was a tendency for an increase in mean energy intake in the milk group compared to the control group (P = 0.07) mean energy intake in the milk group tended).

This discrepancy in results between the Norwegian and Nordic study populations may again be explained by the difference in methods in Sweden and Norway, with regards to the difference in choice of dairy products (low-fat and moderate-fat dairy products versus full-fat dairy products). The choice of low-fat dairy products has resulted in substitution for other foods items in the diet, but has subsequently not led to an increase in mean daily energy intake. The increased dairy intake in the Norwegian milk group has apparently as a consequence led to a reduction in red meat intake, and thereby a probable reduction in saturated fatty acids, which explains why saturated fatty acid intake did not increase significantly and this may also help explain why the mean energy intake did not increase.

6.4 Changes in clinical variables

6.4.1 Body composition

Despite a significantly increased intake of milk protein and calcium, there were no significant changes in body weight, abdominal fat, or the proportion of body fat in the milk group. Thus, we found no support for earlier suggestions that a higher consumption of dairy products, or of calcium in dairy products, would affect body weight or body fat accumulation (56,61,63). The effect of calcium may, however, be beneficial during weight loss, and dairy products may enhance the effect of calcium on weight loss (107), the mechanisms remain largely unclear although there are several suggested mechanisms (105), including increased fecal fat excretion and stimulation of lipolysis and inhibition of lipogenesis (by suppression of 1,25-dihydroxyvitamin D) (94,109). Similar to our results, an intervention during 1 y in young healthy women who increased their intake of dairy products and calcium did
not lead to alterations in body weight or fat mass (110). Also, earlier systematic studies have provided little support for an effect of dairy intake on body weight and fat loss (111,112). The long-term consequences of an increased intake of calcium or dairy products as a part of an ordinary and unrestricted diet on body weight or body fat accumulation remain unclear. Some studies imply that calcium shows its effect only when part of an energy-restricted diet (60), and as this Norwegian study did not result in reduced energy intake, the calcium effect on body weight and blood pressure may not have been evident.

6.4.2 Hypertension

Epidemiologic studies suggest that consumption of milk and milk products is inversely related to the risk of hypertension (66-68), possibly because of an increased intake of calcium and/or magnesium (113) or to the content of protein or specific biologically active peptides (69,114). In apparent contrast with some (115,116), but not all (65), earlier intervention studies, the blood pressure in the Norwegian study population and in the Nordic study population as a whole remained unchanged (Table 5), despite significant increase in calcium intakes and total dairy intake. It has been suggested that the effect of calcium is greater in persons who already have hypertension (117), although a meta-analysis gave no support for this hypothesis (118). The majority of the Norwegian subjects (n=30, 83%) had raised blood pressure at baseline and 60% (n=18) received hypertensive treatment prior to starting the study (11 in the control group and 7 in the milk group, respectively). One could expect that the significant increase in calcium therefore would have a treatment effect on blood pressure specifically. The lack of treatment effect observed, however, may be due to several factors; the small sample size for instance and short intervention period. Furthermore, it has been suggested that there may be a possible threshold for the effect of dairy products and dietary calcium on blood pressure. This threshold has been suggested at a calcium intake of 700–800 mg/d (108). Among the Norwegian study population 41 % (n =7) of the subjects in the milk group had a calcium intake at baseline that exceeded this limit. However, no treatment effect on blood pressure was
seen for those who had a calcium intake lower than 700mg/day. Among the subjects with a low calcium intake, there was, however, a significant treatment effect for waist circumference ($P = 0.023$). No treatment effect was seen for blood pressure in the Norwegian milk group though, as was seen in the Nordic study population as a whole. Again, this may be due to a small sample size in the Norwegian study population. The active form of vitamin D status is important for maintaining calcium levels in the body. The 25-hydroxy vitamin D test is the most accurate way to measure vitamin D status. The result in this study shows that vitamin D status is optimal (above recommended level 75-105 nmol/l) for both the control and milk groups; but there was no significant change in either of the groups after 6 months (Table 8).

Nevertheless, the fact that vitamin D status was optimal indicates that the increased calcium intake is well absorbed.

### 6.4.3 Dyslipidaemia

This study used a validated specific biomarker of dairy fat intake in humans (119-121) to confirm an increased intake in milk fat. Despite this confirmation of increased intake of dairy fat, reflected in a significant increase in 15:0 in cholesteryl esters, no change in the lipid profile was seen in the Norwegian milk group, including LDL cholesterol and apo B lipoprotein.

Contrary to this, in the Nordic study population, an increase in serum cholesterol was mainly seen in the Swedish cohort, which also had the largest increase in milk fat intake among the three countries. There was also a significant increase in serum apo B lipoprotein concentrations within the Swedish milk group only.

Although not included as a criterion for the metabolic syndrome, LDL cholesterol is of interest in this context due to its association with increased CVD risk and as part of the dyslipidaemic triad. Metabolic studies show that most saturated fatty acids, with the exception of stearic acid and short-chain fatty acids raise LDL cholesterol significantly (37). However, it has been suggested that dairy fats solely raise the large and less atherogenic subpopulation of LDL particles (90). Simple LDL measure is
known not to fully predict the cardiovascular risk; small dense LDL particles are considered the most atherogenic. This analysis was not performed in this study, and it is therefore difficult to predict whether there has been a change in the size of LDL particles, in one way or the other. However, when calculating the apoB/apoA1 ratio in the Norwegian subjects, no significant change was observed. The apoB/apoA1 ratio is also linked to an increased risk of CVD and an elevated ratio of apoB/apoA1 (≥0.97 for men and ≥ 0.86 for women) has even been linked to the metabolic syndrome itself (REF). The male subjects in the milk group had a fairly high ratio at baseline (0.97) and this reduced after six months (0.92), albeit non-significantly. The women had a low mean ratio both before and after the intervention (0.79 versus 0.67). It is difficult to draw any conclusions from this observation in this study, as there was no significant increase in saturated fatty acids.

The limited effect on serum cholesterol might in part be related to the choice of dairy products. The Norwegian study population significantly increased the intake of total milk (fluid milk and yoghurt combined) and cheese, but there was only a tendency to a difference in intake of butter compared to the control group. In the Nordic study, the consumption of milk and cheese increased. Butter, which has a higher concentration of milk fat than any other dairy product, seems to have a more pronounced cholesterol-elevating effect than does a corresponding amount of fat from cheese (98-100). Maybe the increased intake in cheese in the Norwegian milk group has buffered the potential cholesterol-elevating effect of butter. Additionally, the lower-fat dairy products as opposed to higher fat alternatives, may have contributed to the lack of effect on cholesterol levels.

Although the intervention meant introduction of many different milk products including fermented products (such as yoghurt), which in some earlier studies (92,93) have shown a potential hypocholesterolaemic effect, we found no significant increase in intake of fermented products (i.e. yoghurt) in the Norwegian milk group. This explains the lack of a hypocholesterolaemic effect in our study.
6.4.4 Insulin resistance

We found a difference regarding changes in the HOMA index, an indicator of insulin resistance, between the groups. This was most likely, however, due to chance, because the main change was an unexpected increase in the control group, whereas no change was seen in the milk group. Furthermore, due to the fact that a range of statistical tests were carried out, it is highly likely that there will be a significant result when the analysis is not adjusted by Bonferroni. Aging per se has no effect on insulin sensitivity, independent of changes in body composition (122). Changes in the HOMA index were unrelated to those of 15:0 and, thus, were presumably not related to changes in milk fat intake. Earlier controlled intervention studies have shown an inverse relation between saturated fat intake and insulin sensitivity (84,86). In these studies the diets were controlled and the difference in saturated fat intake between the test diets, mainly due to a difference in butter fat intake, was larger.

6.4.5 Inflammation, endothelial function and oxidative stress

In women only in the Nordic study, there was a significant increase in leptin during the milk period, and the individual changes in leptin were positively related to the changes in 15:0 in the cholesteryl esters (P = 0.019), which suggests an association with dairy fat or some factor related to dairy fat intake. Whether an increased leptin concentration, in association with an unchanged body fat mass, might indicate a beneficial effect or is a sign of increased leptin resistance is a matter of speculation (123).

An increased proportion of saturated fatty acids in the diet have been related to impaired vascular function (124,125) and markers of inflammation (126). The relatively small and non-significant increase in the intake of fat, and specifically of saturated fat, may explain why there were no changes in markers of inflammation or oxidative stress or indications of impaired endothelial function.
However, the women showed a significant reduction in plasma VCAM ($P = 0.001$). Whether the changes in leptin and VCAM, seen only in women in the Nordic study as a whole, indicate a sex difference in response to an increased intake of dairy products remains to be clarified.

One study has found an association between high cheese consumption and lower levels of PAI 1 in women (59,98), however, the PAI-1 levels did not change significantly in the Norwegian milk group in the present study, and the cheese intake could not be characterized as being high (mean intake 36 g/day ± 23).

There is some research to indicate that some inflammatory markers do improve with increased intake of dairy products, regardless of changes in body weight, this was observed for CRP and adiponectin in a study by Zemel et al. in 2008 (127), however, these markers did not change in this Norwegian study.

### 6.5 Strengths and limitations

#### 6.5.1 Strengths

The strengths include the controlled and randomized design, the follow-up of dietary compliance during the study by the use of the gold-standard weighed dietary intake, and the use of a validated dietary marker in plasma to monitor the dairy fat intake. We also used the gold standard method for analysing body composition, the DEXA scan. The study is also strengthened by the large amount of advanced laboratory analyses. The study was designed to reflect dietary, and related physiologic, changes caused by an increased intake of dairy products in a realistic setting. This type of dietary intervention, in which no instructions were given regarding energy restriction or other dietary changes, giving it a high external validity, is today rare and needed.
6.5.2 Limitations

Among the limitations were the relatively short intervention period of only 6 months and the fact that assessment of dietary compliance, apart from the dairy fat intake, was based on self-reported dietary records (128). One may also argue that our population had a fairly high baseline intake of dairy products and that the increase in the milk group was moderate. The sample size of this Norwegian study was also rather small. The inclusion of participants from 3 countries with partly different background diets in the Nordic study population may be strength of the Nordic study as a whole, but it may also complicate the analysis of the dietary and nutrient intake as the food choices and food culture and not least the composition of the food items differ. So, for this Norwegian study, the dietary analysis is less complicated due to the fact that participants are from the same area with access to the same food items, although the culture and preferences may differ between the individuals.

Future studies may benefit from controlling and defining the dairy intake in more detail to allow for ease of analysis and the overall food intake must be evaluated precisely, maybe even to a greater extent than in this study, by using longer periods of weighed intake, or by providing all food products items for the subjects.

Another aspect of the study which may explain the lack of effect of the intervention was the inclusion criteria chosen. May the subjects included have been too healthy for an effect to be shown? The average number of metabolic syndrome criteria fulfilled was 2.5 and the most frequently fulfilled criteria were enlarged waist circumference and increased blood pressure. The average plasma glucose levels, and HDL cholesterol levels were normal, TGs only slightly elevated. One may therefore speculate that the changes would have been larger if the subjects had had more metabolic abnormalities at baseline and, if the sample size had been larger.

It is interesting how little the overall dietary intake changed during the study. One possible explanation for this may be the fact that this was a study situation and the
subjects were compliant with the protocol and thus were conscious of not changing the overall diet to a large extent.

Milk drinking and intake of dairy products are in many places around the world associated with better health and a healthier lifestyle; such as avoidance of smoking, moderate alcohol consumption, regular exercise and higher social class (94). Another limitation of this study is therefore the fact that these factors were not all adjusted for and not thoroughly evaluated at baseline, although the subjects were instructed to maintain their normal exercise level and lifestyle. This may have influenced the results.
7. Conclusion and future perspective

Although this study cannot verify any clear effect of an increased dairy intake on obesity and blood pressure, it cannot be excluded that such an effect might exist after longer intervention periods of maybe as much as several years and/or if there was a larger difference in intake between the groups. The absence of changes in body weight or body fat during six months suggests the possibility of some restrictive effect on body fat accumulation of an increased intake of milk products. Also, the effect on waist circumference in subjects in the milk group with a low habitual intake of calcium gives some support to the hypothesis of a possible threshold effect. Thus, it cannot be excluded that an effect on body composition might be seen in a population with a lower intake of dairy products at baseline.

This study does not have an immediate or direct impact on any dietary recommendations. However, the study is of value for future research in this field and does indicate that there is a need for larger and longer intervention studies to evaluate the value of dairy products in a healthy diet for individuals with the metabolic syndrome especially, and for a health promoting diet in general. The inclusion of low-fat dairy products as part of a healthy diet as is recommended at present, still has its place. However, it may be of benefit to further advice individuals with the metabolic syndrome of the overall dietary composition with specific emphasis on which other foods to reduce when introducing or increasing dairy product intake.

This study was not, due to the small sample size, capable of discovering clinical significant effects of this intervention. The dairy intake in this study was not large enough to find positive metabolic effects among subjects with only 2.5 criteria for metabolic syndrome fulfilled. In a future perspective, this may imply that to unravel the potential metabolic effects of clinical importance a larger sample size is needed, and possible larger intakes of dairy products are required. However, such a diet may be difficult to achieve within a normal diet in a realistic setting. Moreover, there is a potential for an increase in LDL cholesterol with a higher dairy and saturated fatty
acid intake and subsequently increased risk of CVD. This must therefore be thoroughly deliberated.

Reference List


23. Frayn KN. Metabolic regulation


Appendices

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Appendix 2

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Appendix 8

Overview of Clinical investigations and visit schedule