Body fat distribution, inflammation and cardiovascular disease

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"The more I learn, the more I learn how little I know"

-Socrates

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Abbreviations

ANOVA	Analysis of variance
BMI	Body mass index (kg/m^2)
CT	Computed tomography
CRP	C-reactive protein
CVD	Cardiovascular disease
DXA	Dual energy x-ray absorptiometry
ESR	Erythrocyte sedimentation rate
FFA	Free fatty acid
GI	Glycemic index
HDL	High density lipoprotein
HR	Hazard ratio
HRT	Hormone replacement therapy
ICD	International statistical classification of diseases and related health problems
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
MI	Myocardial infarction
MONICA	Monitoring trends and determinants in cardiovascular disease
MRI	Magnetic resonance imaging
MSCR	Military service conscription registry
RA	Rheumatoid arthritis
RR	Relative risk
SAT	Subcutaneous adipose tissue
SD	Standard deviation
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
VIP	Västerbotten intervention programme
WC	Waist circumference
WHO	World Health Organization
WHR	Waist to hip ratio

Original papers

I. Wiklund P, Toss F, Weinehall G, Franks PW, Nordstrom A, Nordstrom P (2008) Abdominal and gynoid fat mass are associated with cardiovascular risk factors in men and women. J Clin Endocrinol Metab 93 (11):4360-4366

II. Toss F, Wiklund P, Franks PW, Eriksson M, Gustafson Y, Hallmans G, Nordstrom P, Nordstrom A (2011) Abdominal and gynoid adiposity and the risk of stroke. Int J Obes *online publication* 22 Feb 2011 doi:10.1038/ijo.2011.9.

III. Toss F, Nordstrom P, Nordstrom A (2011) Body composition and mortality. Submitted for publication.

IV. Toss F, Nordstrom A, Nordstrom P (2011) Erythrocyte sedimentation rate in young adulthood is associated with myocardial infarction later in life. Submitted for publication.

Abstract

Cardiovascular disease (CVD) is one of the major health issues of our time. The prevalence of CVD is increasing, both in industrialized and in developing countries, and causes suffering and a decreased quality of life for millions of people worldwide. CVD can have multiple etiologies, but the main underlying cause is atherosclerosis, which causes blood clot formation and obstructs vital arteries.

Multiple risk factors of atherosclerosis have been identified, and body fatness is one of the most important ones.

The main aims of this thesis were to investigate the relation between body fatness and: CVD risk factors (*paper I*), incident stroke (*paper II*), and overall mortality (*paper III*). The results showed that abdominal obesity is strongly associated with both CVD risk factors and stroke incidence (*papers I-II*). The results also suggested that a substantial part of the association between increased body fat and stroke can be explained by an increase in traditional stroke risk factors associated with increased body fat (*paper II*). A gynoid fat distribution, with a high share of fat located around the hip, is, on the other hand, associated with lower risk factor levels in both men and women, and with a decreased risk of stroke in women (*papers I-II*). This illustrates the importance of assessing the overall distribution of body fat rather, than solely focusing on total body fatness.

In elderly women, total body fat was found to be associated with increased survival, while abdominal fat moderately increased mortality risk (*paper III*). Lean mass (fat-free mass) was strongly associated with increased survival among elderly men and women (*paper III*).

Erythrocyte sedimentation rate (ESR) is an indicator of inflammation and, possibly, an indicator of atherosclerotic disease. In *paper IV*, the relationship between ESR in young adulthood and the later risk of myocardial infarction (MI) was studied. Results showed that higher levels of ESR were associated with a higher MI risk, in a dose-responsive manner, and was independent of other well-established risk factors.

In summary, both total and regional fat distribution are associated with CVD risk factors and stroke, but do not seem to correspond to an increase in mortality risk among the elderly. Also, inflammation, detected as an increase in ESR, is associated with long term MI risk in young men.

Summary in Swedish - Sammanfattning på svenska

De senaste decennierna har levnadsvillkoren i de industrialiserade länderna förändrats drastiskt. Medicinska framsteg och bättre tillgång till mat har gjort att vår förväntade livslängd är längre än någonsin tidigare[1,2]. Sjukdomspanoramat har förändrats från att tidigare domineras av infektionssjukdomar och bristtillstånd till följd av undernäring till att idag allt mer bestå av sjukdomar relaterade till övervikt[3].

Ett för högt energiintag kan leda till förändringar i blodkärlen, så kallad ateroskleros (åderförkalkning), som leder till stelare kärl och ökad risk för bildning av blodproppar[4-6]. En blodpropp i ett kärl som försörjer hjärtat eller hjärnan kan få allvarliga konsekvenser i form av hjärtinfarkt eller stroke[7,8]. Dessa två ingår i begreppet hjärt-kärlsjukdom, som nu är den vanligaste dödsorsaken i industrialiserade länder[9,10].

Det är sedan länge känt att övervikt i form av ett högt Body Mass Index (BMI) är kopplat till en ökad risk för hjärt-kärlsjukdom[3,11]. Tyvärr är BMI inget idealt mått för att mäta övervikt eftersom det enbart är baserat på individens längd och vikt och därför inte tar hänsyn till fördelningen mellan fett och muskler. Mycket tyder på att en stor andel muskler inte är kopplat till någon ökad risk för hjärt-kärlsjukdom, utan att det är fettet som man borde mäta. Eftersom detta är dyrare och mer komplicerat finns det idag endast ett fåtal studier som mätt fett på ett mer exakt sätt och undersökt kopplingen till hjärt-kärlsjukdomar.

Förutom att den totala mängden kroppsfett är viktig så verkar det dessutom som att fettets placering på kroppen är av betydelse. Fett placerat runt bukorganen, så kallat visceralt fett, tros vara farligare än ytligt fett placerat på höfter, armar och ben[12,13]. Visceralt fett frisätter en högre andel fria fettsyror och inflammatoriska ämnen i blodet än vad ytligt fett gör, vilket kan öka risken för aterosklerosutveckling[14].

Man tror också att det kan finnas en koppling mellan mängden kroppsfett och andra kända riskfaktorer för hjärt-kärlsjukdom. Övervikt innebär ofta högre blodtryck, blodsocker och blodfettsnivåer[6,14]. Livsstilsförändringar i form av förbättrad kosthållning och ökad motion rekommenderas idag till

patienter som bedöms ha en hög risk för hjärt-kärlsjukdom. Genom att undersöka sambandet mellan kroppsfett och andra kardiovaskulära riskfaktorer kan man förbättra kunskapen om hur och varför positiva livsstilsförändringar kan minska risken för hjärt-kärlsjukdom.

Som tidigare nämnts så är ateroskleros en blodkärlssjukdom och förändringar i blodkärlen tycks förekomma hos nästan alla individer[15]. Man trodde länge att fetter i blodet var orsaken till uppkomsten av ateroskleros, men det har visat sig att immunförsvaret också spelar en viktig roll[16,17]. Det verkar som att inflammatoriska ämnen som finns i blodet är kopplade till omfattningen av ateroskleros, åtminstone hos medelålders och äldre individer[18,19]. Om inflammation också är ett tecken på ateroskleros hos yngre är idag inte känt. Genom att tidigt identifiera individer med aterosklerotiska förändringar kan man få bättre möjligheter att sätta in förebyggande behandling. Detta kan minska risken att personen senare drabbas av en stroke eller hjärtinfarkt.

I *studie I* fann vi att kroppsfett, och särskilt bukfett, är starkt kopplat till andra riskfaktorer för hjärt-kärlsjukdom. Det visade sig också att risken för stroke var förhöjd hos personer med mycket bukfett (*studie II*). En stor del av det sambandet verkar bero av kopplingen mellan fett och de kardiovaskulära riskfaktorerna.

När vi i *studie III* sedan studerade sambandet mellan kroppsfett och livslängd hos äldre fann vi att kvinnor med mycket kroppsfett lever längre än kvinnor med lite kroppsfett. Det visade sig också att män och kvinnor med en stor muskelmassa lever längre än de med lite muskelmassa.

I den fjärde studien undersökte vi hur sänkareaktion, ett blodprov som mäter inflammation, är kopplat till risken för hjärtinfarkt. Blodprovet togs hos ett stort antal unga män i samband med mönstringen och det visade sig att ett förhöjt värde ökar risken att drabbas av hjärtinfarkt senare i livet. Utifrån våra resultat så hoppas vi bidra till att man i framtiden kan bli bättre på att identifiera unga personer med en förhöjd risk att drabbas av hjärt-kärlsjukdomar.

Introduction

CVD is now the most common cause of death, and is increasing in incidence at an alarming rate[20]. The global increase in CVD is primarily caused by two factors:

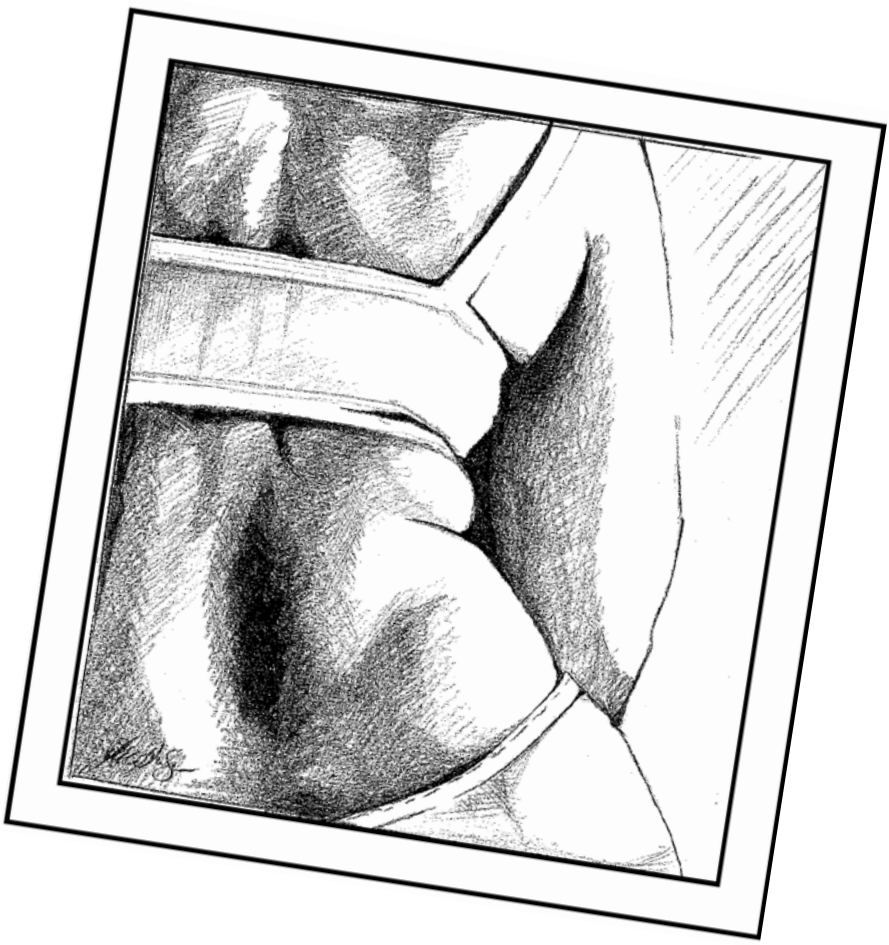
1) An increase in living standards, with a more sedentary life style and an excessive caloric intake, have caused an increase in obesity, which has subsequently been followed by a clustered increase in CVD risk factors[3,6,21]. These factors are associated with an increase in the rate of atherosclerosis formation, the major determinant of CVD[4,17,22].

2) The overall life expectancy is continuing to rise[1,2]. Early mortality, due to malnutrition or infections, has dropped considerably over the last century and advances in the medical field have let more people live longer[3]. Atherosclerosis is a progressive process that, to some extent, occurs in all individuals[4,15]. Therefore, as people live longer, symptoms caused by manifest atherosclerosis become more common.

The rate of atherosclerosis formation is reducible, both by life style and medical interventions. By better understanding the factors that influence disease progression, one can hope to identify subjects in which prevention can hinder the development of CVD.

The aim of this thesis was to create a better understanding of how fatness affects CVD risk factors, incident CVD, and mortality risk. We also aimed to investigate whether inflammation in young adulthood is associated with long term risk of MI.

Obesity



Definitions of obesity

Obesity can be defined as "a condition of abnormal or excessive fat accumulation in where health may be adversely affected"[3]. The overall goal to classifying obesity is to identify individuals and groups that are at an increased risk of morbidity and mortality. Prevention and interventions can then be targeted to individuals or groups with greater accuracy, using predefined criteria.

The World Health Organization (WHO) has chosen Body Mass Index (BMI) to quantify overweight and obesity[3]. BMI is calculated as the weight (kg) divided by the square of the height (m). Overweight is defined as a BMI between 25- 29.9 kg/m^2 and obesity is defined as a BMI ≥30 kg/m^2.

The association between BMI and impaired health is affected by, among others, ethnicity and age. Lower BMI cut-offs has been proposed when assessing risk in subjects with Asian heritage[23], while higher cut-offs might be applicable in older people[24]. There are pros and cons in adapting BMI cut-offs depending on age and ethnicity, and the WHO has thus far recommended the same definitions of overweight and obesity to be used globally, in all adults, and independent of age[3,23].

Central fat, located around the abdomen, is associated with a greater risk of disease than peripheral fat[12,14,25]. To account for this, measures of central obesity have also been developed. The most commonly used measures are Waist Circumference (WC) and Waist to Hip Ratio (WHR). Indication of hazardous abdominal fatness is considered to be: WC>102 cm or WHR ≥0.90 for men; WC>88 cm or WHR≥0.85 for women[25].

More advanced measurement techniques allows for quantification of abdominal and gynoid fat masses. Abdominal fat is considered to be fat located on the abdomen, while gynoid fat is located at the lower limbs and/or around the hip area[26,27]. However, to date, there is no consensus on precise definitions of these fat masses, and there are therefore no specific recommendations on cut-off points.

Epidemiology of obesity

The prevalence of obesity is increasing worldwide and is considered to be the number one preventable cause of illness and premature death[3,9]. By 2005, 1.1 billion adults and 10% of all children were overweight or obese and this figure is expected to continue to rise. North and South America along with Western Europe have the highest proportions of obese individuals, while the prevalence is still relatively low in Asia and Africa[21].

As illustrated in **Figure 1**, in Sweden, there has been a steady increase in BMI over the past 30 years. The average BMI from 1980 to 2008 has risen by 1.66 kg/m² in men and 0.76 kg/m² in women. Also, the majority of men and more than one third of women are now considered overweight or obese[1]. In Sweden, about 14% are estimated to die due to effects of overweight and obesity. Due to the average increase in BMI, the present figure is 3% higher than just 30 years ago. (Estimates from figures provided by the Swedish central bureau of statistics and Banegas *et al.*[28]).

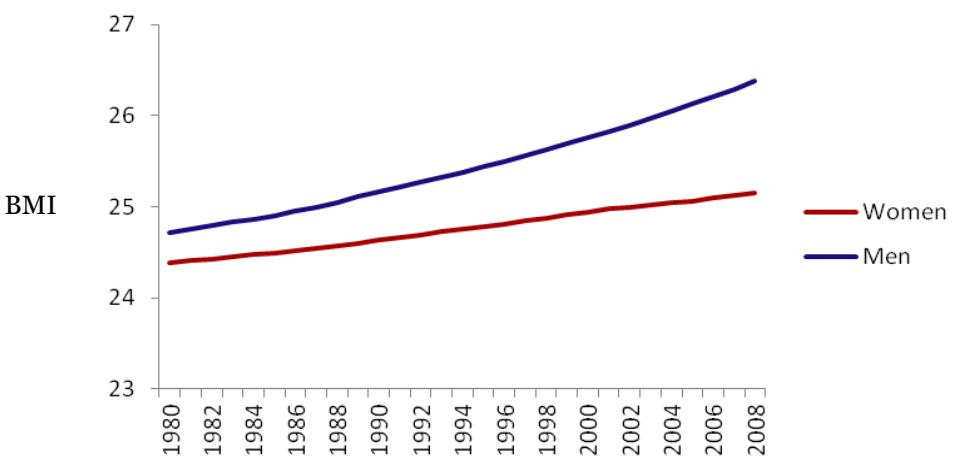


Figure 1. Average BMI in Swedish men and women between 1980 and 2008.

Data supplied by MRC-HPA Centre for Environment and Health, School of Public Health, Imperial College London

Measurement of body fatness

Multiple methods for estimations of body fatness have been developed. The most common methods used in clinical and epidemiological settings include BMI, WC and WHR. For more precise measures of total body fat, dual energy x-ray absorptiometry (DXA) is often used. Abdominal and visceral adipose tissue (VAT) is generally measured by computed tomography (CT), magnetic resonance imaging (MRI), or ultrasound.

Anthropometry

Anthropometric measurements provide approximate estimates of fatness and have the advantages of being quick, cheap, and readily available. BMI is most commonly used to estimate total body fatness and is correlated both to CVD risk factors and CVD[6,29,30]. One of the downsides of the BMI scale is that it does not solely reflect fat, but it is also affected by muscle mass and skeletal weight[3,31]. To measure central obesity, WC, WHR, sub-scapular skinfold, and the ratio of sub-scapular to triceps skinfolds measurements are in practice. They are generally considered better than BMI at predicting CVD risk[30,32,33], but they are poorly reproducible and still only provide crude estimates of abdominal or visceral fat[25].

Dual energy x-ray absorptiometry (DXA)

DXA is a low-dose radiation technique capable of measuring fat mass, fat-free mass (commonly referred to as lean mass), and bone mineral content[34,35]. DXA is particularly good at assessing total body composition, but can also be used to estimate regional fat and muscle distribution[36-39]. New DXA technology is being developed that will be able to distinguish between VAT and subcutaneous adipose tissue (SAT), but currently there is no data available on the accuracy of this method. The main drawbacks of DXA are the lower availability and considerably higher costs than anthropometric measures. The performance characteristics of DXA are covered in the *Materials and Methods* section.

Computed tomography (CT)

CT can accurately differentiate between SAT and VAT and is considered the gold standard for abdominal and visceral fat mass measurements[40,41]. VAT is commonly calculated by a single slice image at the umbilical level[41], but volume can also be estimated from multiple slices[42]. Despite calculations being performed manually, CT has excellent inter- and intra-observer reproducibility[41]. Despite its good performance, CT is not commonly used in

large-scale epidemiological studies due to the radiation exposure during examination, low availability and high costs.

Pathophysiology of obesity

Obesity is a complex disease, with no simple relationship between fat mass and impaired health. Simply focusing on fat mass is often inadequate. Some individuals have a high quantity of fat but low metabolic risk, while others with relatively little fat are at high risk of disease development[22,43]. This variation is largely dependent on two factors, size and location of adipocytes[14,44-47].

Adipocyte size and differentiation.

During positive energy balance, increased storage of energy optimally occurs through the formation of added functional adipocytes through adipogenesis (hyperplasia). If adipogenesis is inadequate, or dysfunctional adipocytes are produced, this can lead to impaired uptake of glucose and free fatty acids (FFA)[48], leading to ectopic lipid accumulation, decreased insulin sensitivity, and potentially type 2 diabetes[14,45,49].

The initial response of adipocytes during a positive energy balance is to increase in size (hypertrophy). This triggers paracrine signaling to produce more adipocytes with the purpose of maintaining adipocyte cell size and physiological functions[46]. Ultimately, whether the net effect to a positive energy balance is adipose tissue hyperplasia or hypertrophy is determined by genetic factors and actions of multiple regulatory proteins[47]. Examples of factors that promote hypertrophy are androgens (testosterone), inflammatory cytokines (IL-6 and TNF-α) and glucocorticoids, while catecholamines, estrogen and very low-density lipoprotein (VLDL) promote hyperplasia[44].

Fat storage

The pathogenesis of metabolic disease is influenced not only by the storage of fat (hypertrophy versus hyperplasia), but also by the location where the fat is stored. Adipose tissue can be divided into two major compartments: SAT and VAT. SAT is located underneath the skin, while VAT is located inside the peritoneal cavity surrounding the peritoneal organs.

The location is probably one of the main reasons why SAT and VAT have different pathogenic potentials. VAT secretes FFA and glycerol into the portal vein, thereby increasing hepatic triglyceride and glucose production,

while promoting dyslipidemia and hyperglycemia. Furthermore, VAT appears to be more metabolically active, with a high lipolytic activity that is less sensitive to the anti-lipolytic effect of insulin. Other factors that are abundantly released from visceral adipocytes include pro-inflammatory cytokines (IL-6 and TNF-α), PAI-1 (decreasing blood clot degradation speed) and angiotensinogen (increasing blood pressure), which all contribute to increased CVD risk[14,44].

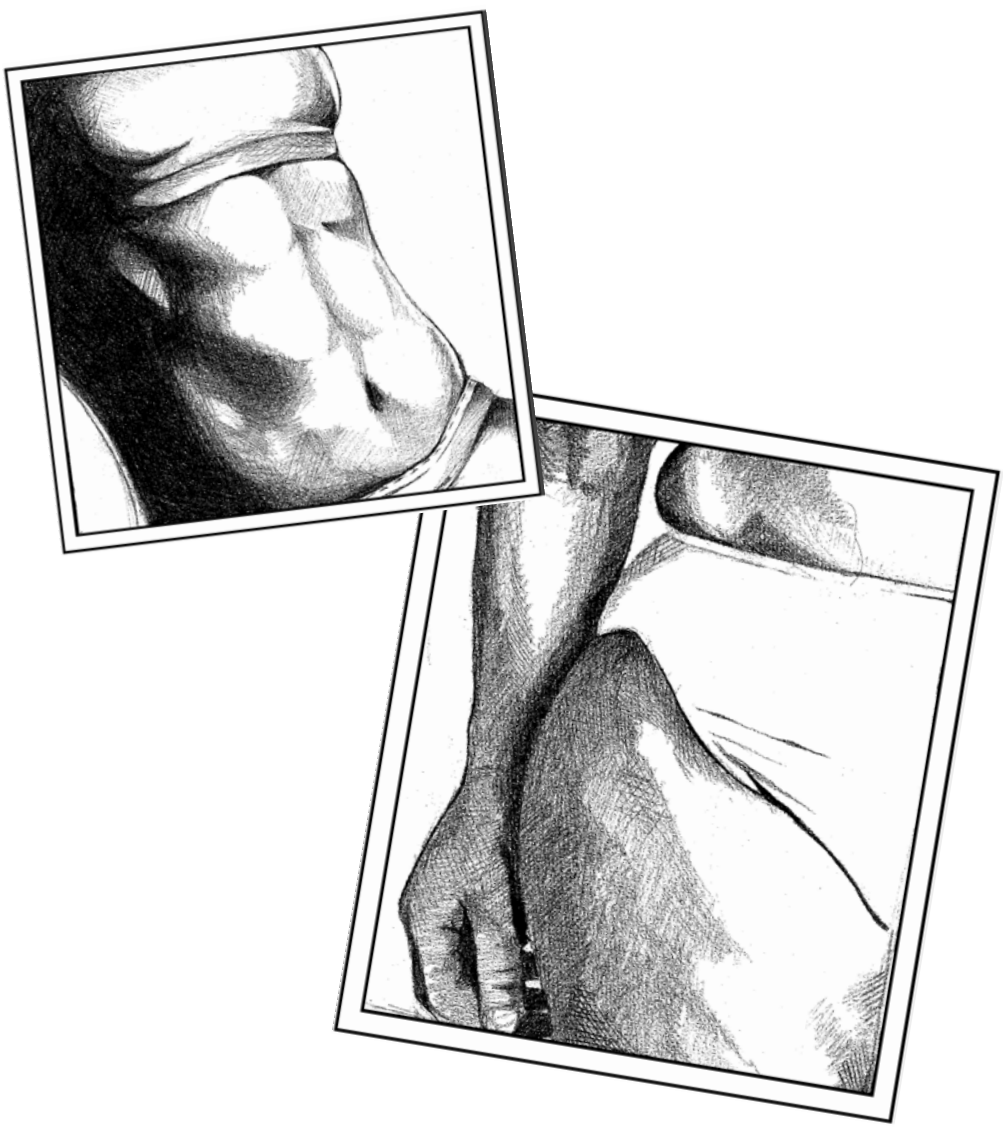
Subcutaneous tissue, on the other hand, is sometimes viewed as protective[12,13,49]. During positive energy balance, small and well-functioning adipocytes can reduce circulating FFAs and thereby decrease the rate of atherogenesis[14,50]. Even though SAT is not as harmful as VAT, it is still correlated to CVD risk factors such as diabetes, hypertension and hypertriglyceridemia[49].

Obesity and cardiovascular risk factors

The etiology of CVD is multifactorial and influenced by several factors in addition to obesity. Hypertension, dyslipidemia, impaired glucose tolerance, smoking, heredity, age and sex are some of the strongest risk factors of CVD[51,52] and obesity is associated with several of these. The role of hypertension, dyslipidemia, and impaired glucose tolerance in atherogenesis is further discussed in the *atherosclerosis* chapter.

Most individuals that develop CVD have multiple CVD risk factors. The clustering of risk factors observed in many individuals is generally referred to as the "metabolic syndrome". There are several different definitions of the metabolic syndrome, but most are based on the presence of (abdominal) obesity, an unfavorable blood lipid profile, elevated blood pressure and insulin resistance[53]. Whether the use of these definitions improves clinical decision making compared to considering each risk factor individually is uncertain and has been a matter of debate[54].

Body composition



Differences in body composition by age

There is great variability in the individual aging process. Changes in body characteristics are dependent on a large variety of factors besides chronological age[55], but the same trends in body composition are seen in most individuals. For instance, a gain in body weight is commonly observed from age 20 to 60, and is primarily attributed to gains in adipose tissue mass[56-58]. The gains in body weight are usually followed by a modest decrease after the age of 60, which is attributed largely to a decline in muscle mass[57-59]. After the age of 75, there is also a marked decrease in total body fat[58].

In aging, there is a redistribution from peripheral to central fat and from subcutaneous to visceral fat. This age-associated decline in subcutaneous fat depots is accompanied by fat accumulation outside adipose tissue, e.g. in muscle, liver and bone marrow[56]. Changes in body composition are caused by a decrease in physical activity[60] and by hormonal[61,62] and nutritional changes[63].

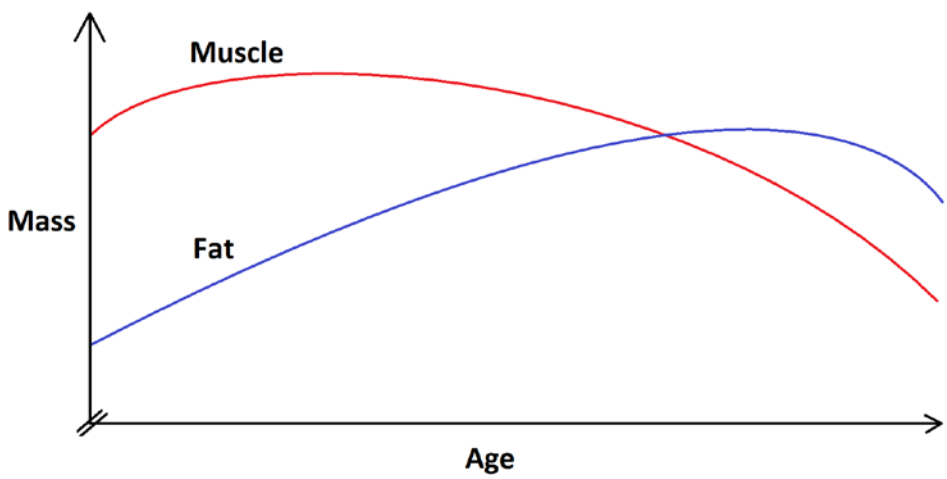


Figure 2. Diagram of fat and muscle mass from adolescence until old age.

Differences in body composition by sex

Sex differences in body composition are evident, beginning at the fetal stage[64] and becoming more pronounced during puberty[64,65]. Men have greater total lean mass and bone mineral mass, and less fat mass than women[64-66]. These differences are consistent throughout life and remain significant, even after adjusting for differences in height[64].

Not only do men and women differ in body composition, but they also differ in their distribution of fat and muscles. In women, the majority of fat is stored peripherally while men accumulate a greater part around the abdomen[64-66]. Men have greater upper body muscle mass, while the difference in lower limb muscle mass is less pronounced[66,67]. Due to these differences, the WHO suggests using different thresholds for WHR and WC between men and women[25]. BMI average, however, is similar in both sexes, so use of the same thresholds are acceptable.

Sex differences in body composition and fat distribution are largely due to actions of sex steroid hormones. In men, a reduction of free testosterone is associated with an increase in fat mass and reduction of muscle mass[62,68]. Correspondingly, estrogen deficiency in women is associated with an increase in visceral fat distribution[61]. Even though women have a higher body fat percentage than men, they still have a lower risk of CVD[1,61,69]. One possible explanation for this is that men, on average, have more visceral fat than women[49]. Also, hormonal differences are likely to play a role since estrogen has been proposed to reduce atherogenesis[61,70] and hormone replacement therapy (HRT) has been suggested to reduce the risk of coronary heart disease in middle-aged postmenopausal women[71]. The protective effect of estrogen is attributed to actions on the vascular endothelial cells. Activation of the estrogen receptor promotes production of endothelial nitric oxide, leading to smooth muscle cell relaxation and an inhibition of smooth muscle cell proliferation, ultimately resulting in a decrease in vascular tonus[70,72]. Even though there are positive theoretical effects of estrogen, it should be noted that HRT does not change or may even possibly increase the risk of CVD in the elderly[71,73]. Because of the increased risk for breast and endometrial cancer after HRT[74], this treatment is currently not recommended as primary prevention for CVD[75,76].

Positive effects of body fatness

In my view, the general public apprehension is that fat is harmful and should be restricted as part of our diet. Most people consider themselves to have a high fat percentage and would like to reduce their content of body fat. Although this loss of fat would likely be beneficial, it is also important to recognize that not all fat is negative. On the contrary, fat is, in fact, crucial for our survival.

Throughout human history, weight gain and fat storage have been viewed as signs of health and prosperity. In times of frequent food shortage, holding a sufficient energy buffer has been essential for survival. The overwhelming majority of calories are stored in the body in the form of fat. Even though muscle-derived proteins can be used as a long-term energy source, the subsequent loss of muscle mass is almost always disadvantageous. The loss of muscle mass during starvation is less in individuals with larger fat reserves, and, in general, is less in females than in males[77]. The higher ability for females to survive during times of famine are also well-documented[78,79].

Due to a limited food supply in much of human evolution, there has been a selection towards adiposity-promoting genes. The ability to store fat has not only been vital to one's own survival, but it is also important for reproduction and for passing on one’s genes. Female fertility is particularly dependent on a certain amount of body fatness, as famine or anorexia nervosa are known to disrupt the menstrual cycle[80]. Fat stores are also beneficial for pregnancy outcome, where low maternal weight and poor weight gain during pregnancy are known to result in low birth weight in infants[81].

Another important body function for survival is the immune system, which requires substantial energy to maintain optimal function. Negative energy balance reduces immune function and increases susceptibility to infections[82,83]. On the other hand, by mechanisms not yet fully understood, immune functions also seem to be impaired in obese individuals[83].

Even with no apparent food deficit, underweight individuals have a decreased life expectancy compared to those of normal weight[11,84]. Smoking and pre-existing disease likely contribute to the increased mortality associated with leanness, but a substantial part of the association remains unexplained.

Noticeably, most positive effects associated with fat are achieved by going from a state of underweight to normal weight, while health benefits going from normal weight to overweight are rare.

Negative effects of body fatness

As mentioned in the previous section, maintaining sufficient calorie intake has been a huge health issue during human evolution. Thus, individuals with a pronounced ability to accumulate fat had a competitive edge, so genes promoting fat accumulation were favored by evolution. Now, as industrialization has lead to a significant decrease in the need for manual labor and food is abundant in large parts of the world, the pendulum has shifted so that obesity is becoming the main health concern. Take, for example, the Pima Indians, who have excellent genetics for fat accumulation[85]. In their traditional way of living, with a high degree of physical activity and low caloric diet, obesity was not a major health issue. However, today, when most of these individuals live a modern western lifestyle, obesity and type II diabetes have reached epidemic proportions. In contrast, the Pima Indians who choose to live a more traditional lifestyle have significantly lower BMI, plasma cholesterol, and type II diabetes[86].

Excessive fat accumulation is associated with numerous negative outcomes. The causality of some of these associations, in particular CVD, will be described elsewhere in this thesis. A selection of diseases associated with obesity are shown in **Table 1**.

Table 1. Relative risks (RR) of health problems associated with obesity[a]

Greatly increased risk (RR>3)	Moderately increased risk (RR 2-3)	Slightly increased risk (RR 1-2)
Diabetes mellitus type 2	Coronary heart disease	Cancer (breast-, endometrial- and colon cancer)
Insulin resistance	Hypertension	Reproductive hormone abnormalities
Gallbladder disease	Osteoarthritis (knees)	Polycystic ovary syndrome
Dyslipidemia	Hyperuricaemia and gout	Impaired fertility
Breathlessness		Low back pain
Sleep apnea		Increased risk of anesthetic complications
		Fetal defects associated with maternal obesity

[a] All relative risks are approximate

Source: WHO-Obesity: preventing and managing the global epidemic (2000)

Modifying body composition and body fat distribution

In theory, losing total body fat is quite simple. By maintaining a negative energy balance, the body will eventually lose weight, most of which will probably be from fat depots. A negative energy balance can be established by either increasing total energy expenditure or by reducing calorie intake. Body fat distribution is a bit more complex, since it is attributable to both genetic, endocrine, dietary, and lifestyle factors. Some of the determinants of body fat distribution will be discussed briefly in this section.

Exercise

Exercise increases calorie consumption, which would lead to a weight reduction. Often, though, an increase in physical exercise leads to a compensatory increase in food intake. Therefore, weight loss attempts with exercise, without dietary restriction, will only have a minor impact on total weight[87].

This does not, however, mean that exercise is ineffective in reducing obesity-related disease risk. In fact, physical exercise will promote muscle build up and will reduce total fat mass if a constant weight is maintained. With increased exercise, there is also a redistribution of fat, with a greater reduction in visceral fat mass[87-89]. However, there appears to be no evidence that one can achieve site-specific fat loss by locally training a certain area. Thus, fat reduction by exercise appears to be due to systemic rather than local effects[90], e.g. doing sit-ups is no better than walking, when trying to reduce abdominal fatness.

Physical fitness is inversely associated with type II diabetes, CVD and mortality. These inverse associations appears to be independent of body weight[91].

Diet

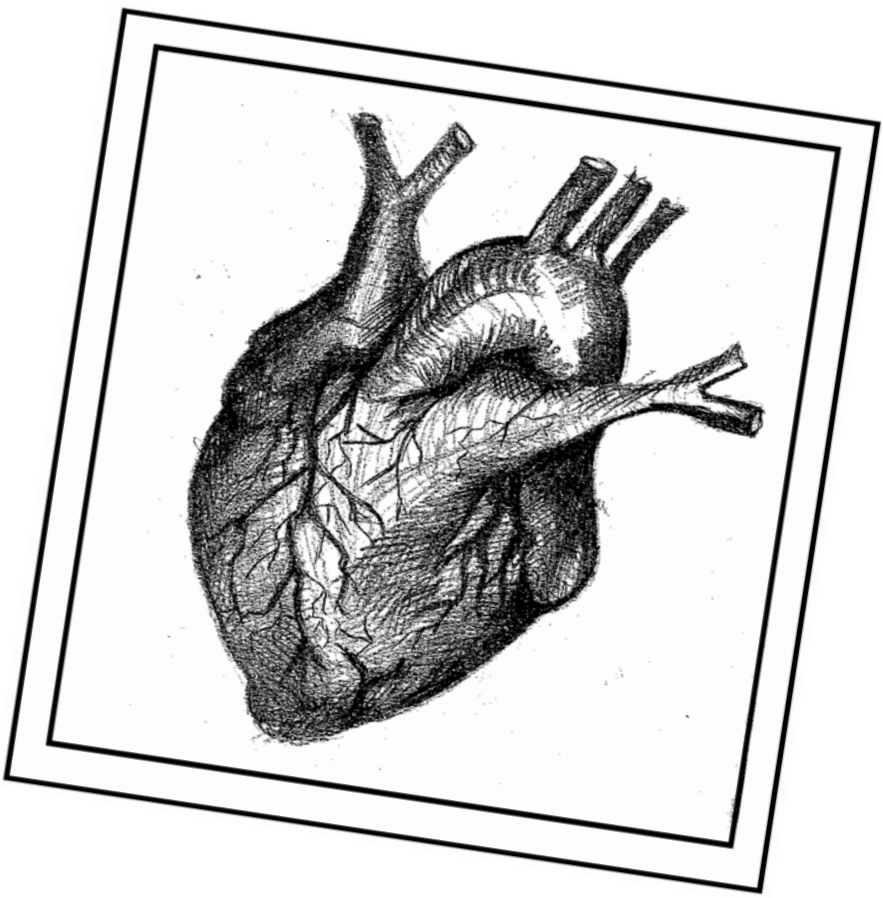
As previously mentioned, restricting energy intake is vital to achieve and maintain weight reduction. Alterations in diet content, in addition to caloric restrictions, have been proposed to make it easier to maintain a negative energy balance. Examples of weight-reducing diets are: low-fat[92], moderate-fat (Mediterranean)[93], high-protein[94], low-carbohydrate[95], and low-glycemic index (GI)[96]. These diets have all shown positive short-term results, but maintaining weight after the initial weight loss has proven difficult on all of these diets. The best diet for healthy obese individuals has been a matter of debate for a long time. The answer to this question will not likely be found in

the near future. Ultimately, it seems that the preferable diet is one that is long-term sustainable, and is a matter of personal preference.

Studying indirect mechanisms of diet can potentially provide new insights. Satiety is one of the factors that contributes to total energy intake. Proteins and fiber-rich foods provide a greater perception of fullness than fat- or simple carbohydrate-rich foods containing the same amount of energy[97]. Carbohydrate rich meals also, at least in the short term, suppress food intake more than high fat meals[98]. Considering that fat is energy-dense and has a weak effect on satiety, decreasing the proportion of fat seems to be a theoretically logical way to reduce caloric intake. Furthermore, as a high intake of saturated and trans-fats raises low-density lipoprotein (LDL) concentrations[99] and increases CVD risk[100], these fats should undoubtedly be restricted to a minor part of our diet. It should, however, be emphasized that satiety is difficult to measure and that the number of studies in this field are limited. Some people have argued that high-fat diets provide greater satiety than carbohydrate rich diets, but they seem to be only supported by indirect evidence of a larger initial weight loss on these diets[101].

For patient groups, there may be other factors, besides weight loss, to consider. For example, in a study of type II diabetes patients, a low-carbohydrate/high-fat diet resulted in lower insulin, higher high-density lipoprotein (HDL) levels, and a greater reduction in visceral fat than with a isocaloric high-carbohydrate/low-fat diet[102]. Thus, for type II diabetes patients, a low carbohydrate diet might result in greater risk reduction than diets containing moderate- to high-carbohydrate content. However, due to a lack of long-term studies, the Swedish Council on Health Technology Assessment has, thus far, concluded that there is insufficient evidence to specifically recommend low-carbohydrate/high-fat diets for diabetic patients[103].

Atherosclerosis



Introduction

Atherosclerosis is a vascular-altering process, present in almost all adult individuals[15]. The extent and severity can vary greatly between individuals and the progression of disease is influenced by a large variety of risk factors. Atherosclerosis is often asymptomatic but can, in late stages, cause both chronic and acute blood flow restrictions, resulting in impaired tissue oxygenation and permanent organ damage. Diseases caused by vascular occlusion, such as myocardial infarction, stroke, angina pectoris, and intermittent claudication all have atherosclerosis as their most common etiology. Given the significance of these diseases, prevention of atherosclerosis should be a top priority for public health care systems.

Lipid metabolism

The retention of lipids within the arterial wall constitutes a vital step in atherogenesis. This section will give a brief presentation of human lipid metabolism (**Figure 3**).

Lipids are an umbrella term that includes triglycerides, phospholipids and cholesterol. Triglycerides are mainly used in the body as energy substrates, while phospholipids and cholesterol are important in cell membrane synthesis, and in other cellular processes[104].

During digestion, lipids pass through the endothelial cells of the small intestine and enter the lymph in the form of chylomicrons[105]. Chylomicrons primarily contain triglycerides, but also a small proportion of phospholipids and cholesterol[106]. The chylomicrons pass through the thoracic duct and enter the blood stream at the junction between the jugular and subclavian vein. The chylomicrons are, after a meal, at a high concentration, but are rapidly removed from the circulating blood. Adipocytes and skeletal muscle cells produce lipoprotein lipase (LPL), which hydrolyzes the triglycerides of the chylomicrons. This releases fatty acids and glycerol, most of which are subsequently absorbed by the adipocytes and muscle cells[107].

The triglycerides stored in adipocytes can be released back into the blood stream, in the form of FFA, through activation of e.g. hormone sensitive lipase[108]. The FFA binds to albumin and can be used as energy substrate by most cells. In the liver, FFA gets repacked into lipoprotein particles by the hepatocytes and get released again in the form of VLDL[109]. Adipocytes and muscle cells can, by secretion of LPL, absorb the triglycerides stored in the lipoprotein particles[110]. As the lipoprotein particles lose their triglycerides they become denser. The lipoproteins listed in the order of density:

chylomicrons, VLDL, intermediate density lipoproteins, LDL, and HDL. The lipoproteins role as risk factors of atherosclerosis will be further discussed in the next section.

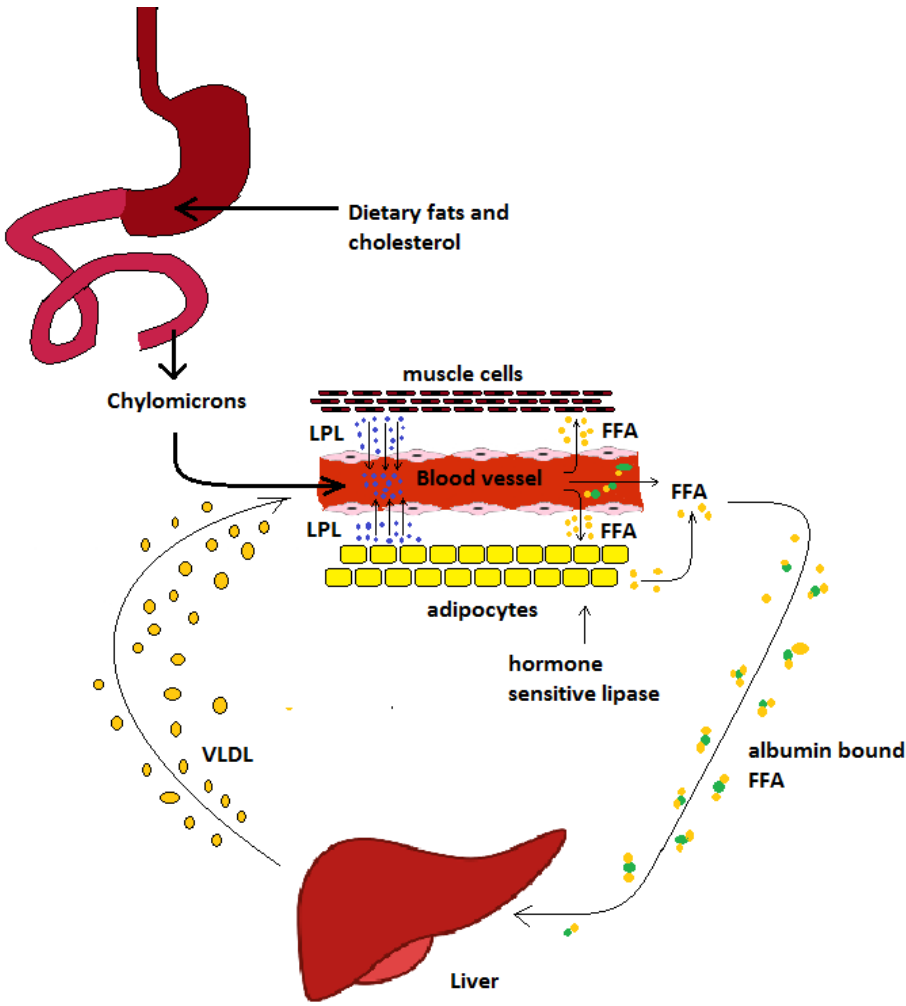


Figure 3. Summary of pathways in lipid metabolism.

Pathogenesis of atherosclerosis

Atherosclerosis is a progressive disease that is characterized by the accumulation of lipids, fibrous elements, and activated immune cells in large- and medium-sized arteries[4,5,16,17]. The mechanisms discussed in this section are illustrated in **figure 4**.

Atherogenesis refers to the development of atheromatous plaques in the luminal side of the artery. The arterial endothelium is a selective barrier that is normally impermeable to immune cells. However, in response to stresses like dyslipidemia, hypertension, or inflammation, the endothelial cells express adhesion molecules that attract monocytes and T-cells, thereby promoting their passage through the endothelial lining. The monocytes then transform into macrophages in response to macrophage-stimulating factors produced by the activated endothelial cells[4,5,16,17].

Parallel to white blood cell recruitment, circulating LDL and other apolipoprotein-B-containing proteins passively diffuse through the endothelial cell junctions, become oxidized, and are trapped within the intimae. When exposed to oxidized LDL or other antigens, macrophages and T-cells answer by secreting pro-inflammatory cytokines, thereby further promoting immune cell activation. The LDL-derived lipids are eventually phagocytized by macrophages, which have no way to fully break down the oxidized lipids. One way they overcome this is to dispose lipids by secreting ApoE, which promotes the efflux of cellular cholesterol back into the blood stream. However, most macrophages eventually become packed with cholesterol and are transformed into foam cells[4,5,16,17].

During atherogenesis, smooth muscle cells migrate from the tunica media to the intima, where they proliferate[111]. In the intima, they produce extracellular matrix molecules, such as elastin and collagen, and form a fibrous cap that covers the plaque. Foam cells accumulate beneath the fibrous cap and when they eventually die, they release lipids that accumulate extracellularly. The body has no efficient way to take care of these cellular and lipid waste products, and, with time, this leads to the formation of a lipid-rich pool called the necrotic core of the plaque[4,5,16,17]. Fibroblasts around the necrotic core produce dense connective tissue (sclerosis), making the artery stiff and unyielding. Later, calcium salts accumulate in the connective tissue, leading to hard calcifications that can make the artery into a rigid tube[112].

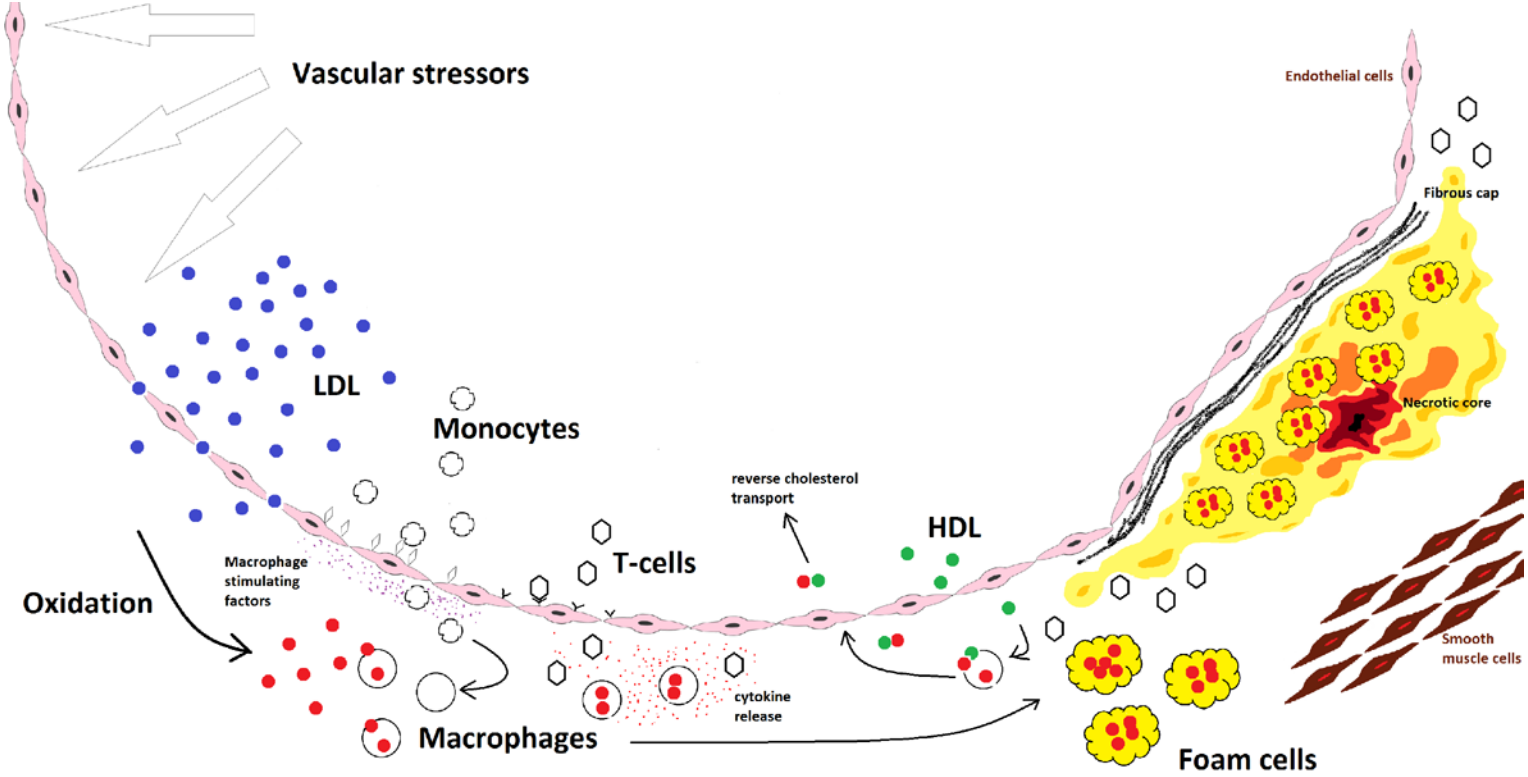


Figure 4. Basic mechanisms involved in atherogenesis.

Link between cardiovascular disease and atherosclerosis

Atherosclerotic plaques are generally asymptomatic, but when they grow large they can cause flow-limiting stenosis that leads to tissue ischemia. This is most noticeable in times of increased oxygen demands. The most serious consequences of atherosclerosis occur at the time of plaque rupture. What causes the rupture is not fully understood, but it appears that it is the composition rather than sheer size of the plaque that is of importance. Increased extensibility, due to less collagen and smooth muscle cells, and a high share of macrophages and extracellular lipids appears to make the plaque more prone to rupture[113,114]. The composition of the plaque can be favorably altered by CVD risk factor control[114].

At times of plaque rupture, material from the plaques' core become exposed to circulating blood and triggers thrombosis formation[4,5,16,17]. The thrombus can then occlude blood flow at the site of origin, or travel downstream and occlude more distal arteries, as a so-called embolus. Ischemic stroke and MIs are perhaps the most feared consequences of thrombi formation. An MI is generally caused by a local thrombi formed at the site of plaque rupture within one of the coronary arteries[7,115]. An ischemic stroke, on the other hand, is also often caused by an embolus formed at a proximal location[8,116].

Risk factors

There are numerous risk factors for atherosclerosis, but this section intends to cover only those relevant to the papers included in the thesis. *Obesity* and *inflammation* are covered in their respective sections.

Lipoproteins

Lipids are hydrophobic, and are therefore not soluble in blood and are mainly transported in lipoprotein particles. In these particles, hydrophilic groups, such as phospholipids and apoproteins, are directed outwards, while triglycerides and cholesterol esters are carried inside.

By synthesis of LPL, cells are able to assimilate triglycerides and cholesterol from the lipoprotein particles, making them smaller and denser. The second most dense of the lipoproteins are the LDL. At high levels, and particularly when small, the LDL particles tend to leak between endothelial vascular cells in the larger vessels and get retained within the intimae. LDL retention, together with immune cell accumulation and activation (as previously discussed), constitutes the first steps of atherogenesis[4,5,16,17].

In epidemiological studies LDL[117], oxidized LDL,[118] and total cholesterol[119] are strong predictors of ischemic heart disease. Also, lowering LDL by use of statins significantly decreases CVD risk[119-122], further supporting the causal role of LDL cholesterol in atherogenesis.

HDL, on the other hand, can reduce atherogenesis by a process known as reverse cholesterol transport[17]. This process refers to the cholesterol uptake by HDL, from foam cells within plaques, and the re-transportation of cholesterol back to the liver. HDL and the HDL/total cholesterol ratio are both negatively associated with the risk of coronary artery disease[119]. However, drugs that aim to raise HDL levels have not been as successful as the LDL-lowering statins. This is likely because there are many types of HDL, so an artificial raise in one type may not be sufficient to improve clinical endpoints[17].

Elevated levels of serum triglycerides, which are also used to screen for dyslipidemia, are associated with increased risk of CVD[123,124], and possibly more so in women than in men[125]. Triglycerides are associated with a detrimental composition of lipoproteins[126,127] and the imparted risk of triglycerides, therefore, diminishes when accounting for multiple other CVD risk factors. Even though triglycerides, themselves, might not be a causal

factors in atherogenesis, the risk associated with elevated triglycerides have been found to be approximately equal to that of LDL[123].

Elevated blood pressure

The pressure within blood vessels puts a continuous strain on artery walls. As discussed previously, an irritating stimulus, such as high pressure, makes endothelial cells more prone to allow passage of LDL particles and immune cells to the intimae[4,5,128]. Therefore, an increase in blood pressure speeds up atherogenesis. Both diastolic, which better reflects the average blood pressure, and systolic pressure, which better reflects the maximal strain, are undisputedly associated with increased CVD risk[52,129-132]. For a long time, clinicians searched for a threshold for increased CVD risk that could be used to define hypertension, but today most evidence is pointing towards a continuous association between CVD risk and hypertension[130]. The definition of hypertension is not solely based on risk, but rather to a point at which the benefits are likely to outweigh the disadvantages of treatment. Hypertension is currently defined by WHO as a diastolic blood pressure greater than or equal to 90 mmHg and/or a systolic blood pressure greater than or equal to 140 mmHg[131].

Elevated blood glucose and diabetes

Diabetes is a disease with multiple metabolic abnormalities, including hyperglycemia, dyslipidemia, hypertension, and coagulopathy. Even though diabetes is primarily characterized by an elevation of blood sugar, a considerable share of the CVD risk associated with diabetes is likely caused by factors other than hyperglycemia[133]. Nevertheless, strict glucose control has been shown to reduce macro vascular complications, such as MI[134] and stroke[135]. The harmful effects of hyperglycemia are hypothesized to originate from overproduction of a superoxide in the mitochondrial electron transport chain, which only occurs when the glucose concentration exceeds a certain threshold level[136]. Excess superoxide production ultimately causes an alteration in gene expression in, among others, endothelial cells and macrophages. These alterations result in an increased vascular permeability, increased coagulability and vascular tonus, and production of pro-inflammatory cytokines[136].

Link between atherosclerosis and systemic inflammation

T-cells and macrophages are abundant in atherosclerotic lesions[137]. They produce pro-inflammatory cytokines, thereby contributing to lesion growth and disease aggravation[16]. Most cytokines exert actions only locally, but some pass through the endothelium and into the blood stream for a more systemic effect. Interferon-γ, TNF-α, and IL-1 are produced within atherosclerotic plaques, but are also produced in response to infection and in fat depots. These factors, in turn, induce production of IL-6, which promotes the production of acute phase reactants from the liver, including c-reactive protein (CRP), amyloid A, and fibrinogen[5]. The amplification of these factors at each step of this cascade makes the end products easy to detect in serum. ESR is affected by, among others, fibrinogen and immunoglobulin concentration, and is thus an indirect measure of the end products of the inflammatory cascade[138-140].

What comes first; inflammation or atherosclerosis?

CRP[18,141-145], amyloid A[143], ESR[19,146-148] and fibrinogen[142] have all been shown to be associated with coronary heart disease, but as these were epidemiological studies, one can only speculate as to the mechanism behind the associations. One possibility is that the inflammatory markers reflect the extent, and perhaps also the degree, of atherosclerosis within arteries. A second possibility is that these markers identify individuals with more active immune systems, and who are therefore also more prone to develop atherosclerosis.

There is indirect evidence supporting both theories. Large-scale Mendelian randomization studies have not found any association between genetically elevated CRP and coronary artery disease, after controlling for other risk factors[144,145]. These results indicate that an elevated CRP does not cause atherosclerosis, but instead implies that an elevated CRP can be caused by atherosclerotic lesions.

On the other hand, conditions with chronic inflammation, such as rheumatoid arthritis (RA)[149,150], lupus erythematosus[151], and psoriasis[152] are all associated with an increased risk of developing coronary artery disease, independently of traditional risk factors. A study on patients with RA found that their risk of MI was normal, until the debut of their disease, when suddenly there was a marked increase in MI risk[150]. Findings from this study support the hypothesis that inflammation causes atherogenesis.

If systemic inflammation is a causal factor of atherogenesis it opens the possibility to reduce risk by treatment with anti-inflammatory drugs.

Myocardial infarction

MI is a major cause of morbidity and mortality worldwide[7]. Atherosclerotic plaque rupture causing epicardial artery occlusion is the most common cause of MI[7,153]. Coronary artery spasm, emboli, or dissection are less common and account for approximately 10% of all MI events[7]. Around 90% of all MIs are estimated to be attributable to modifiable risk factors, such as smoking, dyslipidemia, hypertension, obesity, and diabetes[51].

The current Swedish guidelines for MI diagnostics are based on the elevation of troponins, with either a 50% increase or decrease of two consecutive troponin values within a six-hour interval. At least one of the troponin values has to be above a predefined threshold, which varies depending on the equipment used. For diagnosis, there should also be at least one of the following signs present: typical symptoms, development of a pathologic Q-wave, development of ischemic ECG-changes, or imaging diagnostic evidence of newly developed loss of viable myocardium[154].

Stroke

Annually, 15 million people worldwide suffer from stroke[155]. Of these, approximately 12% die during the first month. Of the survivors, 70% are left with an impaired work capacity and 30% need assistance with self care[69,156]. The disease burden is enormous.

Stroke is defined as "rapidly developing clinical signs of focal (or global) disturbances of cerebral function lasting more than 24 hours (unless interrupted by surgery or death), with no apparent cause other than vascular origin"[157]. Even though symptoms are similar, the etiology of stroke varies greatly. Approximately 85% are caused by cerebral vessel occlusion while about 15% are caused by intracerebral hemorrhage[8,156]. Strokes caused by vessel occlusion can be further subdivided into thrombotic or embolic stroke, depending on the site of clot formation. The two main sources of embolism are carotid stenosis and atrial fibrillation. Intracerebral hemorrhage are often caused by vascular malformation, minor aneurysms, or are secondary to ischemic stroke events[8].

Due to the heterogeneity of stroke etiology, the risk factors vary depending of stroke subtype. Hypertension is the risk factor that contributes to the largest numbers of stroke events[158], while the highest relative risks attributable to

single risk factors are atrial fibrillation and carotid stenosis[69]. Diabetes, smoking, hyperlipidemia are also well-established stroke risk factors[69,159]. Altogether, modifiable risk factors have been calculated to account for 60-80% of the strokes in the general population[159,160].

The rationale for cardiovascular risk prediction

Atherosclerosis is a slowly-progressing chronic disease. The rate of progression can be substantially reduced by improvements in CVD risk factors. Positive life style changes can be recommended without the need for risk calculations. However, drugs that aim at reducing CVD risk cost money and often have unwanted side effects. It is therefore rational to, as early as possible, indentify subjects that have a steep angle of disease progression and are likely to develop CVD. These individuals are likely to benefit the most from preventive actions. This concept is illustrated in **Figure 5**.

Most risk estimates calculate the CVD risk on a 10-year basis[52,161,162]. These estimates work well in older populations, but might make clinicians underestimate the need for prevention in younger adults[163]. This is due to the fact that the absolute 10-year CVD risk is often low in young individuals, despite presence of multiple CVD risk factors. These individuals have an increased life-time risk of CVD and will likely benefit from early prevention. At present, there is insufficient epidemiological data for creation of reliable life time risk charts, but there are indications that such charts would improve clinical decision making, especially in younger individuals[163].

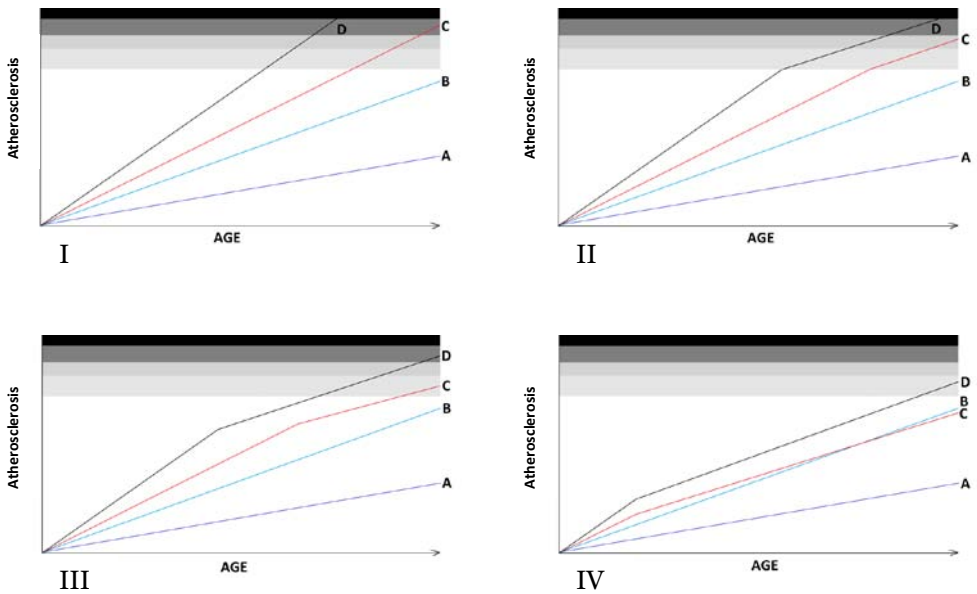
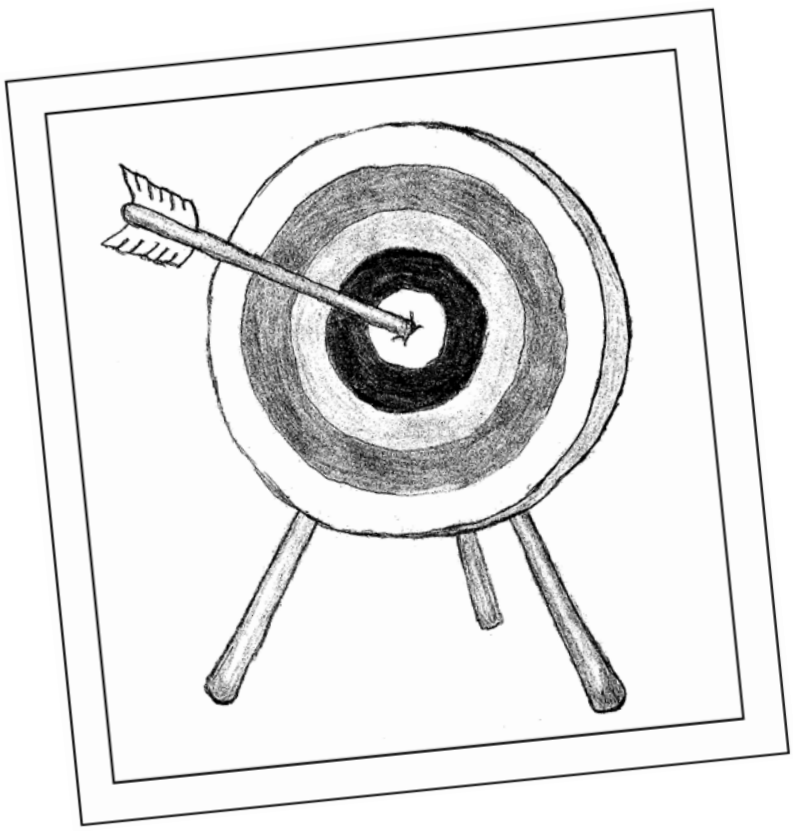


Figure 5. Hypothetical scheme of atherosclerosis development in four individuals (A-D) with different rates of disease progression. The shaded area represents the point at which symptoms of atherosclerosis begins to occur.

I. Disease progression without intervention
II. Disease progression with intervention at debut of symptoms
III. Disease progression with intervention at detection of high 10-year risk of CVD
IV. Disease progression with intervention at early detection of high lifetime risk of CVD

Rationale and aims



The rationale for this thesis

Obesity is a highly prevalent condition, and clearly associated with negative health outcomes e.g. CVD. Much of the current knowledge of obesity associated risks are based on large scale longitudinal studies using anthropometric measures to define obesity. The main drawback of these measures is that they only provide approximate estimations of what is believed to be the primary cause of risk, which is body fat. Experimental data also strongly indicate that the properties of fat cells vary depending on site of location. The risk associated with body fatness might therefore also be dependent on the fat distribution. This has gained support in epidemiological studies using anthropometry, but yet there is insufficient data from studies using more precise measurements of body fat.

In summary, anthropometry enables researchers to investigate associations between obesity and disease in large population based cohorts. However, there is an evident need for verification of their results by use of more precise techniques to quantify body fat and body fat distribution.

Atherosclerosis is the major determinant of CVD and during the past decades it has become evident that the immune system plays a key role in atherogenesis. Markers of inflammation have been found to predict both atherosclerosis and end point disease, such as MI and stroke, in middle age and older populations.

Atherosclerosis is a progressive disorder, and that the rate of progression can be slowed down by risk factor control. Therefore, it is of great interest to study whether inflammation is related to end point disease also at younger ages, where early preventive measures can have a big impact on lifetime CVD risk.

Aims

The overall aim of this thesis was to investigate how body fatness is associated with CVD risk factors, incident CVD, and mortality risk. Another aim was to investigate whether inflammation in young adulthood is associated with risk of MI.

Specific aims:

Study I

- To investigate the cross-sectional association between regional fat masses, measured by DXA, and CVD risk factors.
- To investigate whether physical activity is associated with regional fat masses.

Study II

- To investigate the longitudinal associations between regional fat masses and stroke.
- To investigate whether potential associations between regional fat masses and stroke are dependent on CVD risk factors.

Study III

- To investigate the longitudinal associations between regional fat masses, lean mass and mortality in elderly.

Study IV

- To investigate the longitudinal association between ESR and MI in young men.
- To investigate whether a possible association between ESR and incident MI is dependent on CVD risk factors such as BMI and blood pressure and/or diseases at baseline

Materials and Methods

The papers in this thesis are all based on matching of databases. Here follows a brief description of these databases:

The body composition database

Body compositions, as measured by DXA**,** had been reported since 1991 at the Sports Medicine Unit, Umeå University, Sweden. By the end of 2006, 5,782 subjects had performed a full body scan, and were thus included in the database. Of these, 64% were women and 36% men. The mean age was 43 years (range 2 - 89 years). Reasons for DXA measurements were analyzed for all subjects measured during 2005 and 2006. The most common causes were previous fracture (29%), a general fear of osteoporosis (23%), and previous or present corticoid-steroid therapy (20%). The most common cause of corticoid therapy was rheumatic disease (64%). Overall, 15% of all subjects were not referred and were thus involved in different projects at the Sports Medicine Unit.

Data from the body composition database were used in *study I-III*.

The MONICA database

The WHO initiated the MONICA project (MONitoring trends and determinants In CArdiovascular disease) in 1982. Incidence of stroke were registered, according to strict criteria, at multiple centers throughout the world[157]. The original project ended in 1995, but still continues as a regional project in Västerbotten and Norrbotten, Sweden. The project is population-based, which means that all events, not only those that are treated at a hospital, are registered. Stroke events are registered according to international statistical classification of diseases and related health problems (ICD) criteria in all subjects aged 25-74 years[164]. The ICD criteria are based upon the WHO definition of stroke. Sub-classifications of stroke included subarachnoid hemorrhage, intracerebral hemorrhage, brain infarctions, and unspecified stroke[164].

Data from the MONICA database, on incident strokes, were used in analyzes in *study II*.

The VIP database

The Västerbotten Intervention Project (VIP) is an ongoing project that aims at reducing morbidity and mortality from CVD and diabetes in Västerbotten, Sweden. All residents aged 40-, 50- and 60-years old are invited to enroll. Until 1995, residents at the age of 30 were also able to participate[165]. The project involves a systematic risk-factor screening, followed by individual counseling. By 2007, over 85,000 individuals had participated in the program. Participants visited their health care center following an overnight fast. Height and weight were measured, and BMI was calculated. Blood pressure was measured by a mercury sphygmomanometer, in supine position after a five-minute rest[165]. An oral glucose tolerance test was performed, according to the WHO standards[166]. Subjects with known diabetes mellitus or a fasting glucose exceeding the criterion for diabetes did not undergo the oral glucose tolerance test. A reflotron analyzer (Roche Diagnostics) was used until 2004 to analyze blood lipids and plasma glucose. From 2004 onward, plasma glucose was measured by a Hemocue analyzer (Quest Diagnosis). Participants also answered an extensive lifestyle questionnaire[165].

Data from the VIP database were used in *study I-II*.

The Swedish cause of death registry

The Swedish cause of death registry is administrated by the Swedish National board of health and welfare. The registry includes, among others, information on date, time and primary cause of death of all deceased Swedish citizens. Due to low autopsy rates (currently less than 20%[167]), but also other factors, the information on cause of death are often uncertain[168,169], while data on date of death are highly reliable.

Data from the Swedish cause of death registry was used in *study III*.

The Military Service Conscription Registry (MSCR)

Until recently, military conscription was mandatory for all Swedish men. At enrollment, tests were performed by the Swedish military service administration, in order to find an appropriate position for each individual. Exemptions from enrollment were rare. Foreign citizenship, a severe chronic medical condition, or a documented handicap were the only reasons accepted for nonparticipation[170].

At enrollment, subjects went through highly-standardized intelligence and physical tests. All conscripts were seen by a physician, who diagnosed any disorders according to ICD criteria. Weight, height, and blood pressure were measured, and venous blood samples were taken. The erythrocyte volume fraction was analyzed by the microhematocrit method[171] and ESR by the Westergren method[172].

Data from the MSCR was used in *study IV*.

The National hospital discharge registry

The National hospital discharge registry is administrated by the Swedish National board of health and welfare and has existed in its present form since 1987. The registry covers all discharges from public hospitals in Sweden. The accuracy of this registry is dependent upon diagnosis, but since almost all MI patients are treated at a hospital and strict diagnostic criteria are applied, the statistics on MI are considered to be of high quality[173].

Data on incident MI from the National hospital discharge registry was used in *study IV*.

Study populations

For *study I-III*, the cohorts were gathered from the *body composition database*. Characteristics of the various cohorts are shown in **table 2**.

Study I investigated the cross-sectional association between body fat distribution and CVD risk factors. Out of all subjects in the body composition database, 592 (70% women), subsequently participated in the VIP and were thereby included in *study I*.

In *study II*, we examined the relationship between fat distribution and stroke incidence. All subject in the body composition database that were between the age of 40 and 75 years were considered for inclusion. After exclusions of subjects that had suffered a stroke prior to study start, the cohort consisted of 2,751 subjects, among whom 73% were women. 760 of these subjects had, either prior to or after study start participated in VIP. Information from these subjects on smoking, diabetes and hypertension was used to test if these variables affected the body composition-stroke associations.

Study III investigated body composition in relation to mortality in elderly men and women. All subjects in the body composition database at the ages of 65 or older were included in this study. In total, this cohort consisted of 921 subjects (79% women).

In *study IV*, 457,263, 18-year-old men, which were enrolled for conscription between 1969-1978, were included from the MSCR. After exclusions for missing data, extreme data points, mortality, or emigration, 433,456 subjects remained available for further analysis. The primary purpose of this study was to investigate the relationship between ESR and MI. In a subset of 22,490 individuals, where smoking habits had been reported, a secondary goal was to determine if smoking had an impact on the association between ESR and MI.

	Study I		Study II		Study III		Study IV
	Women	Men	Women	Men	Women	Men	Men
No. of participants	417	175	2 013	738	728	193	433 456
Age (years)	47	45	57	55	73	72	18
Weight (kg)	68	82	68	82	65	77	68
Height (cm)	165	179	163	177	160	173	179
BMI (kg/m^2)	25	26	26	26	25	26	21
Total fat mass (kg)	25	20	26	22	26	22	-----
Abdominal fat mass (kg)	1.6	1.5	1.5	1.5	1.6	1.7	-----
Gynoid fat mass (kg)	2.7	1.9	2.7	1.9	2.6	1.9	-----
Lean mass (kg)	-----	-----	-----	-----	39	55	-----
ESR (mm/h)	-----	-----	-----	-----	-----	-----	3.4
Follow up time (years)	-----	-----	9		10	8	35

Table 2. Mean values of a selection of baseline characteristics of the cohorts in *study I-IV*.

Dual energy X-ray absorptiometry (DXA)

In *study I-III*, DXA (GE Lunar, Madison, WI, USA) was used for fat and lean mass measurements. Until 1998, subjects were measured by a DPX-L. From 1998 onward, a Lunar IQ was used.

In DXA analysis, the body is subdivided into three compartments: fat mass, bone mineral content, and fat-free mass.

DXA accurately estimates total fat mass (r^2=0.84-0.91, compared to hydrostatic weighting[34]), abdominal fat mass (r^2=0.80-0.92, compared to MRI[36] or CT[37]), VAT (r^2=0.61-0.72, compared to MRI[36] or CT[37,38]) and muscle mass (compared to MRI[39]). Discrepancy between investigators is low (intra-class correlation=0.989[37]), and inter-person reproducibility, i.e. scanning the same person multiple times, results in very little variation in measurements (coefficient of variation=1-5%[36]). The radiation exposure during a full body examination is less than that accumulated over one day of normal background radiation[35].

In *study I-III* we determined the regional fat masses from full body scans (**Figure 6.**).

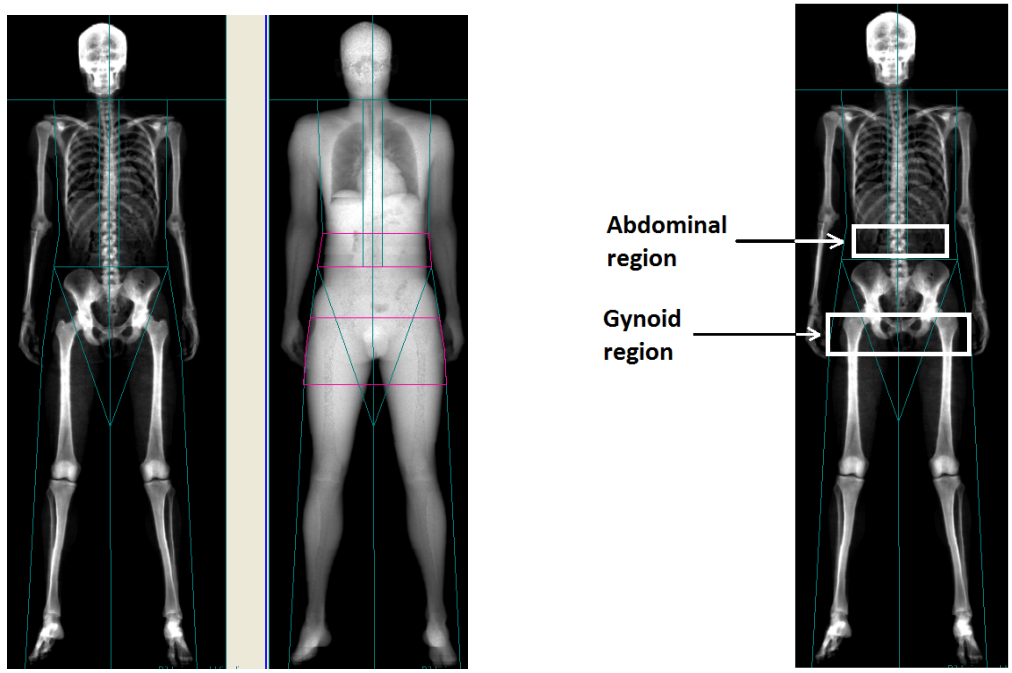


Figure 6.

A. Skeletal and soft tissue image from DXA full body scan **B.** Definitions of abdominal and gynoid fat regions

Erythrocyte sedimentation rate (ESR)

ESR measures the distance a column of anticoagulated blood will fall during one hour. The premise behind this is that negative charges on the surface of red blood cells make them repel each other making the sedimentation rate slow. When, however, red blood cells are in contact with positively-charged macro molecules, i.e. inflammatory proteins such as immunoglobulines and fibrinogen, the negative repelling forces becomes neutralized and results in an increased sedimentation rate. An increased ESR is, therefore, an indirect measure of inflammation[138-140]. In addition to inflammation, ESR is also affected by the concentration of red blood cells. All analyzes involving ESR were therefore adjusted for the erythrocyte volume fraction.

In *study IV*, ESR was analyzed by the Westergren method, which is considered to be the reference method[172].

Statistical methods

Study I. Bivariate correlations between the adiposity measures and the continuous CVD risk factors were assessed by the Pearson coefficient of correlation. Differences between multiple groups were analyzed by analysis of variance (ANOVA) and Bonferroni´s post hoc test. The relationship between the fat measures and the categorical CVD risk factors were determined by logistic regression.

Study II. Differences between two groups were analyzed using the Student´s T-test for independent samples. The longitudinal associations between the normal scores of the adiposity measures and incident stroke was determined by Cox regression.

Study III. Differences between two groups were analyzed using the Student´s T-test for independent samples. Cox regression was used to assess the relationship between the normal scores of the adiposity measures and mortality. To investigate possible non-linearity, subjects were divided into quartiles for each fat measurement. U-shaped trends were tested by adding the square of the adiposity estimate to the Cox regression model.

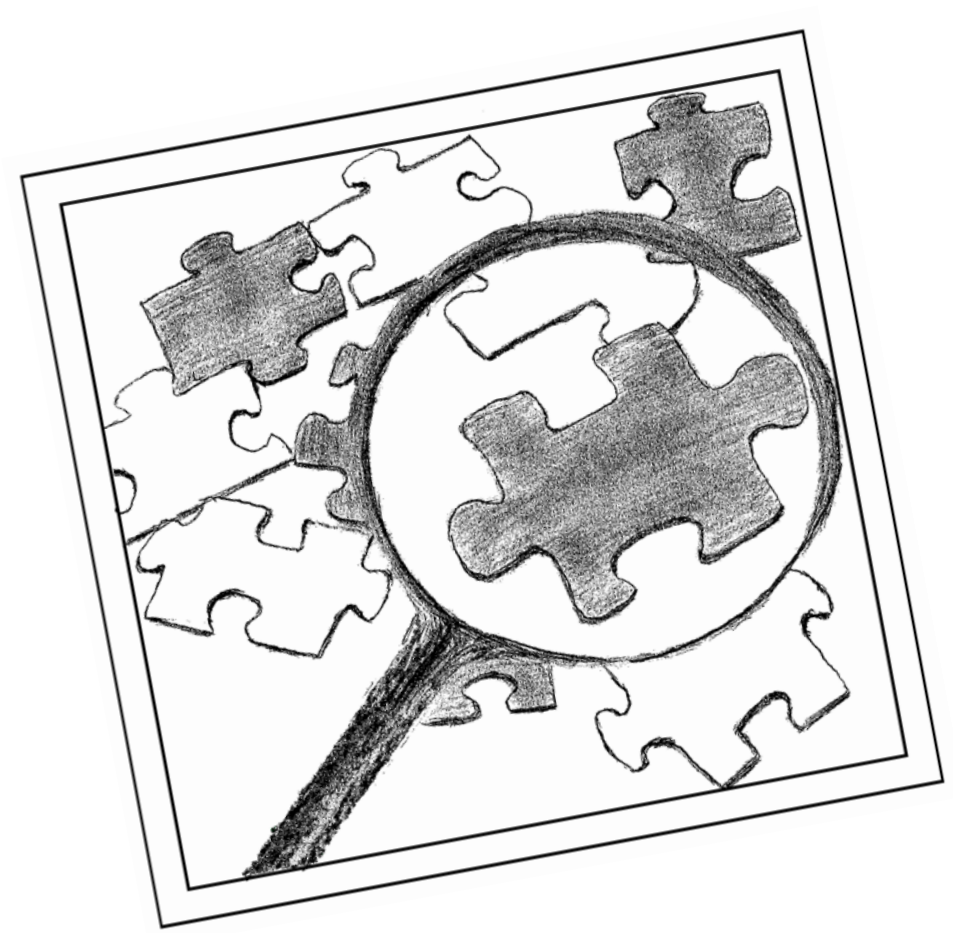
Study IV. Differences between two groups were analyzed using the Student´s T-test for independent samples. Normal distribution of ESR was obtained by a natural logarithm transformation. The longitudinal association between ESR and MI was determined by Cox-regression. To investigate a possible dose-response relationship between ESR and subsequent MI, subjects were split into 10 groups, according to their ESR value. Group 1, with the lowest ESR values, was considered reference.

In study I-III, analyzes were performed separately for men and women. In all studies, a p-value less than 0.05 was considered significant. SPSS (version 15 and 17, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Ethics

All studies (*Study I-IV*) were approved by the Regional Ethical Review Board in Umeå, Sweden. *Study IV* was also approved by the Swedish National Board of Health and Welfare.

Results



Results Study I

Abdominal and Gynoid fat mass are associated with cardiovascular risk factors in men and women

This study was a cross-sectional investigation of regional body fatness, body fat distribution and a selection of CVD risk factors. Bivariate correlations between the measures of regional adiposity and the CVD risk factors are shown in **table 3**. In both men and women, the estimates of adiposity were generally positively correlated with systolic and diastolic blood pressure and with serum triglycerides ($p<0.05$). Gynoid fat was positively correlated with many of the risk factors, while the ratio of gynoid to total fat mass was generally negatively correlated with the same factors. Physical activity was negatively correlated with all measures of fat masses ($r=-0.24$ to -0.13 $p<0.05$), and positively correlated with gynoid fat distribution ($r=0.12$ to 0.19 $p<0.05$).

In subsequent analyzes, the continuous CVD risk factors were categorized according to widely used definitions of impaired glucose tolerance, hypertension, hypercholesterolemia and triglyceridemia. Analyzes of the associations between the adiposity measures and the categorized CVD risk factors were adjusted for age, time between DXA measurement and participation in VIP, smoking and physical activity. These analyzes showed that triglyceridemia and hypertension were associated with close to all measures of adiposity in both men and women ($p<0.05$). Impaired glucose tolerance was associated with the measurements adiposity in men, but not in women. Hypercholesterolemia was not significantly associated with any of the adiposity measurements ($p>0.05$). In general, abdominal fat mass showed the strongest association to the CVD risk factors. These results are presented in **table 4**.

	Total fat	Abdominal fat	Gynoid fat	BMI	Abdominal fat/gynoid fat	Abdominal fat/total fat	Gynoid fat/total fat
Males							
Fasting plasma glucose	0.15	0.20[a]	0.14	0.20[a]	0.15	0.03	−0.18[a]
2-h blood glucose	0.08	0.11	0.08	0.00	0.11	0.11	−0.08
Systolic blood pressure	0.18[a]	0.27[b]	0.17[a]	0.20[a]	0.28[b]	0.17[a]	−0.22[b]
Diastolic blood pressure	0.27[b]	0.34[b]	0.25[b]	0.28[b]	0.27[b]	0.12	−0.28[b]
Triglycerides	0.25[b]	0.31[b]	0.24[b]	0.24[b]	0.20[a]	0.06	−0.23[a]
Cholesterol	-0.05	0.09	−0.07	−0.07	0.32[c]	0.33[c]	−0.09
Physical activity	-0.19[a]	−0.24[b]	−0.20[a]	−0.21[a]	−0.14	−0.03	0.19[a]
Females							
Fasting plasma glucose	0.21[c]	0.22[c]	0.20[c]	0.24[c]	0.18[c]	0.09	−0.19[c]
2-h blood glucose	0.12[a]	0.16[b]	0.12[a]	0.11[a]	0.15[b]	0.13[a]	−0.08
Systolic blood pressure	0.23[c]	0.27[c]	0.23[c]	0.20[c]	0.21[c]	0.15[c]	−0.16[c]
Diastolic blood pressure	0.14[b]	0.20[c]	0.14[b]	0.11[a]	0.20[c]	0.17[b]	−0.12[a]
Triglycerides	0.26[c]	0.33[c]	0.22[c]	0.22[c]	0.32[c]	0.21[c]	−0.27[c]
Cholesterol	0.01	0.06	0.02	−0.02	0.12[a]	0.14[b]	−0.03
Physical activity	-0.14[a]	−0.15[a]	−0.13[a]	−0.08	−0.10[a]	−0.06	0.12[a]

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

Table 3. Bivariate correlations between measures of adiposity, fat distribution and CVD risk factors in men and women.

Explanatory variables	IGT	Hypercholesterolemia	Triglyceridemia	Hypertension
Males				
Abdominal fat mass	2.69[a]	1.25	3.37[b]	2.63[b]
Gynoid fat mass	2.07[a]	1.10	2.10[a]	2.15[b]
Total fat mass	2.15[a]	1.06	2.22[a]	2.13[b]
BMI	2.52[a]	1.24	1.99	3.05[b]
Abdominal fat/gynoid fat	1.33	1.23	1.77[a]	1.32
Abdominal fat/total fat	0.90	1.28	1.04	1.01
Gynoid fat/total fat	0.52	1.00	0.42[b]	0.61[a]
Females				
Abdominal fat mass	1.25	1.09	1.97[b]	1.77[c]
Gynoid fat mass	1.04	1.09	1.41	1.57[b]
Total fat mass	1.20	1.05	1.66[a]	1.68[c]
BMI	1.32	1.02	1.54[a]	1.64[c]
Abdominal fat/gynoid fat	1.48[a]	1.11	2.19[c]	1.60[c]
Abdominal fat/total fat	1.27	1.20	1.71[b]	1.36[a]
Gynoid fat/total fat	0.71	1.01	0.49[c]	0.62[c]

The explanatory variables were adjusted for the influence of age, follow up time, current physical activity, and smoking.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

Table 4. Odds ratios of the CVD risk factors per standard deviation (SD) of the measures of adiposity.

Results Study II

Abdominal and gynoid adiposity and the risk of stroke

This study was an investigation of the longitudinal relationship between measures of adiposity and incident stroke. During follow up, 91 subjects suffered a stroke. In women, all measures of adiposity were significantly associated with stroke risk (Hazard ratio (HR)=1.33-1.66 per SD increase, $p<0.05$). An abdominal fat distribution was associated with an increase in stroke risk (HR=1.47 $p<0.05$), while a gynoid fat distribution was associated with lower risk (HR=0.72 $p<0.05$). In men, abdominal fat was the only significant predictor of stroke (HR=1.49 $p<0.05$). Results from these analyzes are shown in **table 5**.

In secondary analyzes, we tested whether abdominal fat mass was a predictor of stroke independent of BMI. In this model, abdominal fat mass was significantly associated with incident stroke in both men and women (HR=1.71-1.80 $p<0.05$), while BMI was not (HR=0.80-0.90 $p>0.05$).

In a subgroup analyzes, where data on hypertension, diabetes and smoking was available, the model was adjusted for these variables. The adjustments attenuated the associations between the measures of adiposity and incident stroke, and they now no longer remained significant ($p>0.05$ for all).

	HR	*95% CI*	P-*value*
Women (n = *2013*)			
BMI	1.51	1.13–2.01	0.005
Total fat mass	1.45	1.09–1.94	0.01
Abdominal fat mass	1.66	1.23–2.24	0.001
Gynoid fat mass	1.33	1.00–1.78	0.05
Abdominal/gynoid fat mass	1.47	1.09–1.98	0.01
Gynoid/total fat mass	0.72	0.54–0.96	0.03
Men (n = *738*)			
BMI	1.11	0.81–1.52	0.53
Total fat mass	1.35	0.97–1.87	0.08
Abdominal fat mass	1.49	1.06–2.09	0.02
Gynoid fat mass	1.34	0.96–1.86	0.10
Abdominal/gynoid fat mass	1.00	0.72–1.38	1.00
Gynoid/total fat mass	0.91	0.66–1.25	0.54

Abbreviations: CI, confidence interval; HR, hazard ratio. HRs are presented per s.d. increase in the independent variables. Printed with permission from Int J Obes

Table 5. Associations between the different measures of adiposity and incident stroke in women and men, after adjustments for the influence of age.

Results Study III

Body composition and mortality

This study was conducted to investigate the associations between different aspects of adiposity and body composition in relation to mortality in older subjects. During follow up, 397 subjects (43%) died. In men, there was evidence of a u-shaped association between all of the measures of adiposity and mortality ($p<0.01$). In women, a u-shaped trend was observed for abdominal fat mass, while the other measures of adiposity were negatively associated with mortality (HR=0.82-0.85 $p<0.05$). An abdominal fat distribution was associated with a slight increase in mortality risk in women (HR=1.13 $p<0.05$), but not in men (HR=1.01 $p>0.05$). In both men and women, lean mass was negatively associated with mortality (HR=0.69-0.81 $p<0.01$). These results are shown in **table 6**. The same analyzes were also performed excluding subjects that died within the first two years of follow up. This exclusion had no distinguishable impact on results.

In secondary analyzes the subjects were split into quartiles of each measure of adiposity. In women, the highest risks were generally observed in the first quartile (corresponding to those with the lowest amounts of fat), while in men, the highest risks were observed both in the first and the fourth quartile. For lean mass, the highest risk was observed in the first quartile in both men and women. These results are shown in **table 7**.

Women	p U-shaped trend	HR linear trend	p linear trend
BMI	0.114	0.84	0.003
Abdominal fat mass	0.018	-	-
Total fat mass	0.179	0.85	0.009
Gynoid fat mass	0.214	0.82	0.001
Abdominal/Gynoid	0.104	1.13	0.044
Lean mass	0.512	0.81	<0.001

Men	p U-shaped trend	HR linear trend	p linear trend
BMI	<0.001	-	-
Abdominal fat mass	<0.001	-	-
Total fat mass	<0.001	-	-
Gynoid fat mass	0.002	-	-
Abdominal/Gynoid	0.382	1.01	0.910
Lean mass	0.480	0.69	0.001

Table 6. P for trends and HRs (per SD) of estimates of adiposity in women and men.

Women	BMI		Abdominal fat mass		Total fat mass		Gynoid fat mass		Abdominal/ gynoid		Lean mass	
Quartile	HR	p	HR	p	HR	p	HR	p	HR	p	HR	p
1	1		1		1		1		1		1	
2	0.89	0.455	0.74	0.063	0.82	0.213	0.79	0.146	0.99	0.958	0.668	0.009
3	0.62	0.004	0.79	0.150	0.84	0.267	0.88	0.435	0.90	0.563	0.615	0.002
4	0.78	0.139	0.77	0.119	0.70	0.035	0.62	0.008	1.32	0.099	0.530	0.001
Men	BMI		Abdominal fat mass		Total fat mass		Gynoid fat mass		Abdominal/ gynoid		Lean mass	
Quartile	HR	p	HR	p	HR	p	HR	p	HR	p	HR	p
1	1		1		1		1		1		1	
2	0.39	0.001	1.01	0.967	1.01	0.971	0.75	0.328	1.08	0.791	0.676	0.137
3	0.41	0.001	0.74	0.309	0.70	0.217	1.04	0.886	1.21	0.502	0.564	0.044
4	0.59	0.041	1.52	0.126	1.36	0.248	1.05	0.860	1.09	0.771	0.465	0.010

Table 7. Age adjusted HRs of death by quartiles of estimations of adiposity in women and men.

Results Study IV

Erythrocyte sedimentation rate in young adulthood is associated with myocardial infarction later in life

In this study we examined the longitudinal relationship between ESR and incident MI in young men. During follow up, 8 082 subjects suffered an MI. ESR was significantly associated with incident MI both in the primary model (adjusted for age, EVF, year and place of measurements, HR=1.16 per increase in $\log_e$ESR $p<0.001$) and in the secondary model (further adjusted for BMI, systolic blood pressure, education and disease at baseline, HR=1.18 $p<0.001$).

In secondary analyzes subjects were split into ten groups according to baseline ESR. Subjects with an ESR of 2 mm/h had a significantly higher risk of MI than those having an ESR of 1 mm/h (HR=1.11 $p<0.05$). The risk increase by ESR appeared continuous (p for trend 0.001), and having an ESR of 15mm/h or above was associated with a 68% increased risk compared to those with an ESR of 1 mm/h. These results are illustrated in **figure 7**.

In subgroup analyzes, where present smoking status was know, the impact of smoking on the ESR-MI relationship was tested. Despite the fact that smoking was strongly associated with MI risk (HR=2.30 $p<0.001$), it did not influence the ESR-MI association.

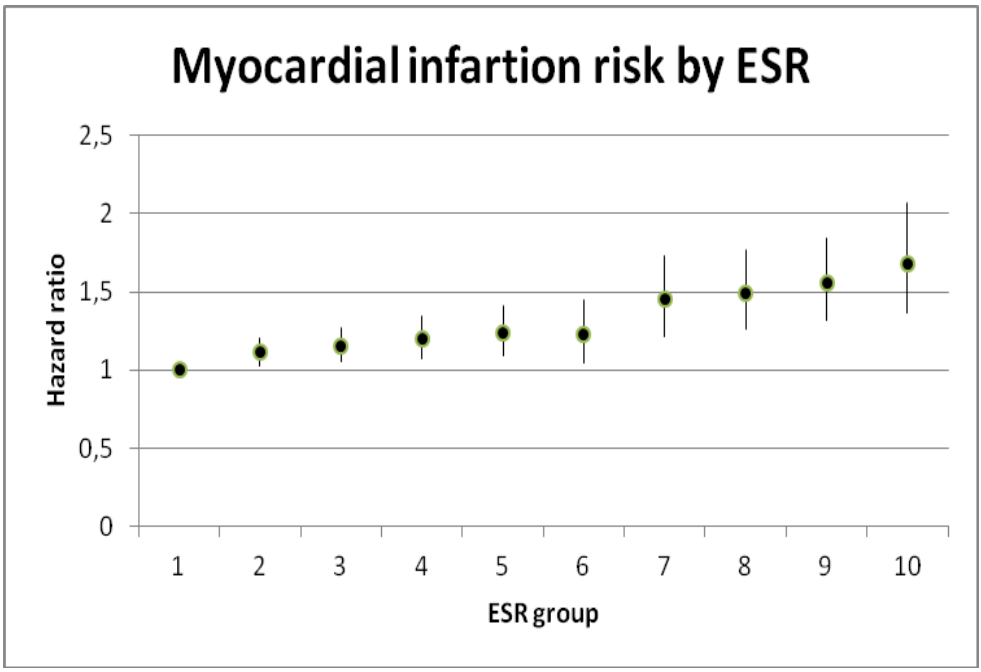
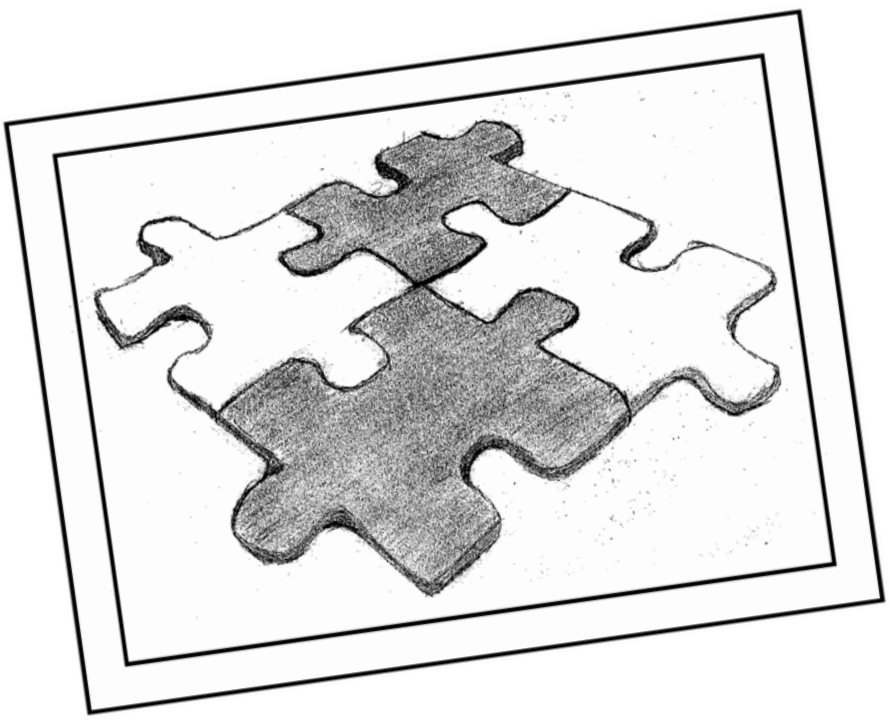


Figure 7. HRs and confidence intervals of MI by groups of ESR.
The model is adjusted for age, EVF, year and place of measurements, BMI, systolic blood pressure, education and diseases at baseline.

Discussion



Regional adiposity and cardiovascular disease

Obesity is a major contributor to the global increase of CVD[3,21]. BMI has been the chosen measure to define the limits of obesity, but research strongly suggests that it is not the best measure to predict CVD risk[29,32]. BMI is only a rough predictor of total body fat, and does not factor in body fat distribution.

Central obesity, and particularly an excessive visceral fat accumulation, has been noted to be particularly deleterious[12,14,30,32,43,49]. The importance of central obesity in CVD risk prediction is recognized by the inclusion of WC, rather than BMI, in attempts made to define the metabolic syndrome[174]. WC provides a better estimate of visceral fat than BMI does[175], and strong epidemiological data shows that WC is associated with other established CVD risk factors[30,176] and with CVD events[25,32].

On the contrary, gynoid fat, localized around the hips, has been suggested to counterbalance the detrimental effects of visceral fat[13]. The ratio of waist-to-hip circumference has, therefore, been proposed as an even better predictor of CVD risk[32]. However, since both of these measurements are affected by skeletal structure and lean mass, the effects of fat masses alone are difficult to interpret.

In *study I*, abdominal fat mass and the ratio of abdominal to gynoid fat mass had the strongest correlations to the CVD risk factors in both men and women. Interestingly, gynoid fat mass was also positively associated with CVD risk factors. This can likely be explained by the high correlation observed between gynoid and total fat mass. When gynoid fat was put in relation to total fat mass, the ratio was negatively associated to risk factor levels. Physical activity was associated with lower body fatness and a favorable fat distribution.

Given the findings in *study I*, it seemed probable that regional fat mass would also be associated with CVD risk. This hypothesis was tested in *study II*, where we investigated the relationship between regional adiposity and the risk of stroke. We found that abdominal fat had the strongest association with stroke in both men and women. As in *study I*, the ratio of gynoid to total fat was associated with lowered risk, but this time only in women. After adjusting for the other CVD risk factors, the associations between the regional fat measures and stroke were no longer significant. We therefore concluded that the association between regional fat mass and stroke was, at least partially, mediated through traditional CVD risk factors.

Results from *studies I and II* are in agreement with studies that have used WC or WHR as their explanatory variables[176-180]. The risks associated with these measures are difficult to compare between studies, since some performed calculations based on quartiles, while others used specific thresholds or continuous values. Studies also vary by follow up time and mean age of participants and are, to some degree, adjusted for different confounders. However, since conclusions from these anthropometric studies are similar to ours, DXA, at present, do not provide sufficient improvements in CVD risk estimations to justify clinical use. However, DXA does provide accurate estimations of fat masses and body composition. Being able to longitudinally follow these measures may, aside from providing risk estimations, also enhance motivation in subjects determined to reduce body fatness.

The conclusion that abdominal fat is associated with CVD risk seems logical, given the atherogenic attributes of visceral fat cells, which is discussed in more detail in the section of *pathophysiology of obesity*. While not protective per se, a gynoid fat storage appears to be associated with lower risk than fat stored at other sites. This has also been noted in other studies using anthropometric measurements[32,181,182]. The protective effect has been suggested to be due to high LPL activity in gynoid fat cells[13], and in summary this theory states that: An excessive postprandial release of FFA, in combination with elevated insulin levels is one of the major factors contributing to the insulin resistance[183]. Postprandial FFAs are not only derived from adipocytes, but also from the fat content of the meal. When LPL in insulin-resistant adipocytes process FFA from chylomicrons, a substantial amount is not esterified and is released into the circulation[183]. Insulin-sensitive cells, such as gynoid adipocytes, do a much better job storing FFA from chylomicron breakdown[184]. Visceral, subcutaneous, and gynoid adipocyte LPL, together with skeletal muscle, all compete for breakdown of meal-derived chylomicrons. Therefore, the LPL activity in different fat depots, to a certain degree, determines the proportion of meal-derived fat that is converted to FFAs. A gynoid fat distribution may therefore be a reflection of high LPL activity in gynoid adipocytes, and is likely associated with lower levels of circulating FFA[13].

Body composition and mortality

CVD (40%), cancer (25%), and respiratory diseases (6%) are the most common causes of death in Sweden[10]. Obesity is strongly associated with CVD, while weakly associated with cancer and respiratory diseases[11]. Given the heterogeneous causes of mortality, the association between obesity and mortality is likely caused by many different mechanisms.

Furthermore, there seems to be an age-interaction between obesity and mortality. In middle-aged adults, the association is J- or U-shaped[11,31], while in the elderly, studies do not agree, since positive, negative, and non-significant associations have all been reported[24,185-187]. Some researchers have proposed that this discrepancy is due to a poor association between fat mass and BMI in the elderly. Although researchers have not found a clear relationship between body fatness and mortality, there is evidence of a protective effect of lean mass[188-192]. Given the difficulty in distinguishing fat and muscle mass by anthropometric measures, assessments need to be made by direct measurement techniques.

In study III, we found that lean mass was negatively associated with mortality in both men and women. Total fat mass was found to be protective in women, while a high proportion of abdominal fat, compared to gynoid fat, moderately increased risk in women. In men, the relationship between both total and abdominal fat mass was U-shaped, with an increasing risk among both lean and obese subjects.

It is difficult to determine whether low muscle (lean) mass is a causal factor contributing to mortality. Muscle mass is strongly associated with muscle strength and declines naturally with age[193,194]. When muscle strength drops below a certain level, mobility is impaired, leading to a loss of independence and a decreased ability to maintain the remaining muscle mass. Individuals with a high muscle mass, thus, fare better with the natural age-associated muscle mass decline and in times of disease when muscle loss occurs more rapidly[195]. Muscle mass is also a good indicator of health, since there is a strong correlation between muscle mass and physical activity[194], another factor that declines in times of disease. To adequately control for this form of reverse causality has proven difficult, since early stages of disease often are undiagnosed. In our study, we tried to control for serious illness by excluding individuals that died during the first two years of follow-up, but these controls did little to change the final results. Despite these efforts to control for reverse causality, we cannot know for sure whether muscle mass, by itself, is protective against mortality, or if it is simply a marker of other mechanisms, such as preexisting disease.

The U-shaped association between total fat mass and mortality in men is probably due to different causes of mortality in lean and obese individuals. Involuntary weight loss and low weight can be caused by respiratory conditions[196] and/or cancer[197]. The association between low fat mass and mortality is therefore not likely to be causal. On the other end of the spectra, the association between high fat mass and mortality is more likely to be causative because of the strong associations between obesity and CVD mortality[11,31], as discussed in more detail previously in this thesis.

Despite having been noted in comparable anthropometric studies[187,198,199], the fact that fat mass is associated with increased survival in women is interesting and still perhaps a bit unexpected. A possible explanation is that individuals with higher levels of body fat have greater energy reserves to use during times of disease, when maintaining a neutral energy balance is difficult. Larger energy reserves would make it possible to survive for a longer period of time with a disease that causes a negative energy balance. This effect could potentially outweigh the increase in CVD risk among very old individuals. Another possible explanation is reverse causality due to preexisting disease, which was discussed in more detail earlier in this chapter. Why high body fatness among elderly was associated with increased survival in women but not in men is difficult to say and warrants further investigation.

Inflammation and cardiovascular disease

All stages of atherosclerosis are characterized by immune cell activation and the immune response appears to be required for atherosclerosis development[16,17]. In response to stressors such as hypertension or high concentrations of LDL, endothelial cells express adhesion molecules that promote immune cell infiltration in the arterial wall. Deposition of LDL underneath the endothelial lining then promotes further immune cell recruitment and activation, ultimately leading to vascular inflammation. Cytokines produced by activated immune cells can be indirectly detected by measuring end products of the signaling cascade. Inflammatory substances have therefore been proposed as markers of atherosclerotic disease [5,16].

CRP[18,141-145], interleukin-6[200], fibrinogen[142,201], and ESR[19,146-148,202] are all positively associated with CVD events in middle-aged populations, where the prevalence of atherosclerotic lesions is relatively high. Early stages of atherosclerosis (fatty streaks) have been detected even in young adults[15], but whether these can be traced by inflammatory markers is, to date, not known.

In *study IV*, the results strongly indicated that a dose-response relationship exists between ESR and MI. This relationship remained even after the adjustment for potential confounders, such as disease at baseline and known CVD risk factors.

Atherosclerotic lesions are the underlying cause of the vast majority of MIs and a previous study have found a strong association between coronary arteriosclerosis and ESR[202]. Our results indicate an association between elevated ESR, as a possible sign of early atherosclerosis, and the development of more advanced lesions later in life. Admittedly, whether atherosclerosis causes inflammation, or if inflammation causes atherosclerosis is not distinguishable in this type of study. The timeline for these events might, at first glance, seem unimportant, but it actually has a high value to the clinical implications of these findings. If elevated inflammatory markers make immune cells more prone to migrate into the arterial intimae and thereby contribute to pathogenesis, one could aim at reducing inflammatory response in high-risk individuals. On the other hand, if inflammatory markers are just indicators of atherosclerosis, one should instead aim at reducing traditional CVD risk factors in the same individuals.

Of the inflammatory markers, CRP has rendered the most scientific interest. Elevated CRP has been shown to be associated with MI risk in a number of large studies[18,141-145]. The elevation of CRP following an acute episode of MI

has also been shown to correlate with prognosis[122,203]. Given these findings, CRP levels have been proposed to be measured for CVD risk factor screening[204], and targeted for drug intervention[121]. Given a causal link, CRP-lowering drugs could potentially be used both as prevention and as an acute treatment in MI.

Genetic studies are helpful to determine causality. Large-scale Mendelian randomization studies have investigated allele variations that are known to be associated with elevated basal levels of CRP[144,145]. These studies have found that CRP, but not genetically-elevated CRP, is associated with MI risk. This indicates that CRP itself does not cause atherosclerosis, but is rather a reflection of the atherosclerotic process. An implication of these findings is that specific CRP-lowering drugs are likely ineffective as a primary prevention therapy. On the other hand, CRP is just one specific molecule associated with inflammation. Other means of reducing inflammation might potentially prove to be more effective.

Statin therapy reduces LDL cholesterol levels, but also has anti-inflammatory properties. In a highly noted study, rosuvostatin was found to reduce CVD risk in individuals with low LDL but high CRP levels[121]. There was no improvement in risk for those with low LDL and low CRP. These results imply that a part of the risk reduction with rosuvastatin can be attributed to its anti-inflammatory properties. However, since statins also have targets not relating to inflammation, there is a need for studies using selective anti-inflammatory drugs to verify the benefits of inflammatory reduction. Currently, there are two ongoing trials that aim to answer this question: one using low dose methotrexate and the other using an interleukin-1 inhibitor[205].

Independent of whether inflammation by itself is a causal factor of CVD, markers of inflammation can potentially be used to predict CVD. CRP has proven to add some predictive value when used in convention with other CVD risk factors[204]. However, it is still not clear whether this relatively small increase in predictive power is relevant to clinical decision-making[163].

In our study, we observed a significant increase in MI risk already at an ESR of 2 mm/h, compared to 1 mm/h (HR=1.11, $p<0.05$). An ESR of 15 mm/h or above was associated with about a 70% increase in MI risk ($p<0.001$). However, in this study, we did not evaluate the robustness of ESR as a screening tool, and given the relatively small differences in absolute risks, it seems unlikely that ESR alone can be used as a screening tool, at least in low-risk populations. Importantly, however, our findings illustrate that CVD

risk can be estimated already in young adulthood and this emphasizes the need for more studies on traditional CVD risk factors in young adults.

Limitations

This section is not intended to give a complete description of all study limitations, but rather to discuss some of most important ones.

Patient based cohorts

In *study I-III*, the majority of participants were patients, which are a specific subset of the general population. It is, therefore, important to determine whether this selection of participants limits the generalizability of our results. In order to assess this, we need to consider what separates this cohort from the general population.

The reasons for DXA measurement were analyzed in subjects measured during 2005-2006. As previously mentioned, the primary reason for admission was not for fat mass assessment, but for measurements of bone mineral density. Previous fracture (29%), a general fear of osteoporosis (23%), and previous or present corticoid-steroid therapy (20%) were the most common causes of admission. The majority of patients (64%) on corticoid therapy had been previously diagnosed with rheumatic disease. Overall, 15% of all subjects were not admitted and were instead involved in different projects at the Sports Medicine Unit at Umeå.

Low bone mineral density is an independent predictor of CVD[206,207]. Given these admission characteristics, we have good reason to suspect that our cohort, on average, had a lower bone mineral density than the general population. Therefore, the incidence of CVD might have been slightly higher in this cohort compared to the general population. The incidence of an outcome is, by itself, not a major factor when trying to determine the generalizability of results. The question is whether certain characteristics of the cohort tend to have an effect on the relationship between the explanatory variables (i.e. fat and lean masses) and the outcome variables (i.e. CVD risk factors, stroke, and mortality).

In theory, the genetic properties of our cohort could cause an interaction. However, it was recently reported that the distributions of genetic risk markers for obesity and diabetes II in our cohort were similar to that in other populations[208], indicating that the cohort was not unique in terms of genetic background.

Another factor to consider is the possible interaction between cortisone treatment, body composition, and CVD risk. Given that the majority of patients on steroid therapy had rheumatic disease, it is most relevant to assess a possible interaction in this group. RA patients appears to have a more central fat distribution, higher fat mass, and lower lean mass compared to age and sex-matched controls[209]. Corticoid therapy was, in one study, associated with increased fat mass[210], while another study found no association[209]. Cortisone treatment did not seem to influence the overall distribution of fat[210,211]. This is somewhat surprising, and might possibly be explained by the relatively low doses of cortisone used in these studies. Hypercortisolism (Cushing's syndrome) is otherwise well known to be associated with an abdominal fat distribution[212]. Both RA and corticoid therapy are independently associated with increased risk of CVD[149,150,213] and RA is also associated with an increased risk of mortality[214].

Since RA, and possibly also cortisone treatment, is associated with both our explanatory and the outcome variables, they could potentially cause our results to appear stronger than they actually are. However, it should be noted that a relatively small percentage of our cohort used cortisone and/or had rheumatic disease, and that the associations between RA (and cortisone) and the explanatory variables appears to be modest. Therefore the higher numbers of these patients in our cohort was not likely to have a substantial impact on our results. Nevertheless, there might also be other potential interactions due to the patient-dominated cohort, so the projection of our results to the general population needs to be made with this in mind.

Possible confounding of infections in study IV

ESR is an unspecific marker of inflammation, and an elevated value can be caused by many other things besides atherosclerosis. One might argue that an elevated ESR can be caused by infectious diseases that also causes an increased risk of MI. In our study we adjusted for many, but naturally, far from all medical conditions. Given that the MI, on average, occurred 30 years after study start, it seems unlikely that an acute infection at baseline could increase MI risk. Chronic or recurrent diseases such as periodontitis or chlamydia pneumoniae infections can cause an elevation of inflammatory parameters and are associated with CVD[215-217]. Whether these, or possibly other infectious diseases, affected the association between inflammatory markers and cardiovascular disease in young adults is not known. An indirect indication that these conditions did not significantly impact the results was that statistical adjustments for other chronic or long term conditions, such as RA and colitis, in our study, did not alter the ESR-MI association.

Generalizability of results to women

In *study IV*, we only studied the relationship between ESR and MI in men, and the results may therefore not be applicable to women. To our knowledge, only two previous studies have examined the relationship between ESR and MI in both men and women. One of them found similar associations in men and women[146], while the other one found associations to be weaker in women than in men[148]. The association between other measures of inflammation and MI appears to be similar in men and women[18,218]. In conclusion, there is insufficient data on the ESR-MI relationship, particularly in younger subjects, for us to be able to speculate whether our results also apply to women.

Clinical implications

In *studies I and II*, we found that fat mass, particularly abdominal fat mass, increased the risk of CVD in middle-aged populations. This emphasizes the need for individuals that have a high risk of CVD to strive towards a better fat distribution and body composition profile. Results from *study I* also shows that physical activity is beneficially correlated with these body characteristics.

For clinical assessment of fat distributions, anthropometric measures are currently sufficient. The development of new DXA-technology which is capable of measuring visceral fat might, however, make DXA a part of conventional CVD risk factor screening in the future.

In *study III*, we found that lean mass was a strong predictor of survival in older people. Therefore, we should be encouraging the elderly population to remain physically active and to avoid a negative energy balance. Whether our results also motivates lean mass measurements by DXA to be used clinically, in older subjects, is difficult to say. To answer this question cost-benefit analyses and comparisons to other measurement techniques are needed.

In *study III*, we also found that high levels of body fat were associated with increased survival in women. These findings are not sufficient to recommend weight gain in the normal-weight elderly, but they imply that we should not strive to reduce body fat at older ages. Voluntary weight change in the elderly is a topic where insufficient evidence exists, and would be interesting to investigate further.

In *study IV*, ESR at a young age predicted MI later in life. This supports the concept that inflammation is part of atherogenesis. These findings might, together with other studies, ultimately lead to improved CVD risk assessments and provide potential new targets for drug development. ESR is currently not sufficiently validated to be included in CVD risk screening, but our findings suggests that CVD risk can be estimated also in young adults. Further studies on this subjects are warranted.

Future research

Our studies have shown that body composition is associated with CVD risk factors and incident stroke. Body composition is determined by a combination of inherent and environmental factors, and it would be interesting to study their individual contributions. Furthermore, the environmental factors can be positively affected. By launching intervention studies targeting diet, exercise, medication, or possibly other mechanisms, we can hope to further the knowledge on how to achieve and maintain positive alterations in body composition in high-risk individuals.

Abdominal fat mass was found to be the strongest predictor of CVD risk factors and stroke in our studies, and constitutes the sum of SAT and VAT. The results from experimental studies indicate that VAT is particularly harmful, so it would therefore be interesting to compare these two fat depots in epidemiological settings. Until now, techniques to adequately measure these fat depots in healthy subjects have either been too expensive or have been associated with a significant exposure to radiation. However, given new progress with DXA technology, measurements of visceral fat in the general population might soon be feasible.

We also found that total fat mass was negatively associated with mortality in elderly women. Whether one should avoid voluntary weight loss, or possibly recommend weight gain in healthy elderly is an issue that warrants further investigation.

In *study IV*, we found that high ESR in young adulthood was a predictor of MI later in life. Whether inflammation is causative or just a marker of disease is a matter of great significance, and clearly more research is warranted on this subject. Further work is also needed to determine whether markers of inflammation have the predictive power to justify inclusion in traditional CVD risk screening.

Results from *study IV* shows that ESR is associated with MI risk several decades before any clinical symptoms occur. It would therefore be very interesting to investigate whether the traditionally-used CVD risk factors, measured during young adulthood, can predict long term CVD risk.

Summary and conclusions

Both total fat mass and regional fat masses were found to be predictors of CVD risk. Abdominal fat mass was generally the strongest predictor of both CVD risk factors and stroke events. A gynoid fat distribution was negatively associated with CVD risk factors in both men and women and had a protective effect against stroke in women. The association between fat masses and stroke was significantly weakened after adjustments for the CVD risk factors, indicating that the effect of fat masses is partially mediated through these factors.

The finding that physical activity was associated with a lower total fat mass and with a beneficial fat distribution indicates that positive changes in body composition can be achieved through life style modification. Lean mass was highly associated with increased survival in older subjects, which emphasizes the importance of physical activity. Interestingly, total fat mass was associated with a decreased mortality risk in older women, and is potentially a sign of health at older ages.

Finally, we found that ESR measured during adolescence is associated with incident MI. ESR is a measure of inflammation and can be a sign of atherosclerosis, since all stages of atherogenesis are characterized by immune cell activity. These findings are in line with previous observations that atherosclerosis is a continuous disease, already present in adolescence.

In conclusion, CVD is the end result of a continuous process of atherosclerosis and can be prevented by a reduction in the rate of atherogenesis. Prevention can be achieved by controlling CVD risk factors, which are correlated with body composition and body fat distribution.

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