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The interaction between skeletal muscle mass and visceral adipose tissue in association with inflammatory- and metabolic biomarkers

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Abstract

Introduction: Obesity has become a global health issue. The reasons for this are complex, but in short, energy-dense food are becoming more accessible and affordable in addition to decreased levels of occupational physical activity over the last decades. Obesity, and especially visceral obesity, triggers mechanisms in the body which lead to a low-grade inflammatory state and imbalances in the metabolism. This state will over time increase the risk of several diseases. Likewise, muscle tissue also effects a variety of biological processes, such as reduced inflammation through secretion of myokines.

Aim: This study aims to investigate how different combinations of skeletal muscle mass and visceral fat mass levels are associated with serum CRP, HbA1c % and serum triglycerides.

Method: The study design for this master thesis is cross-sectional, including data from the population-based sample from the seventh Tromsø study. 3,340 participants were included in this study. The age of the included participants was between 40-84 years. Body composition were measured by dual energy X-ray absorptiometry. Linear regressions were used to investigate the association between body composition and inflammatory- and metabolic biomarkers.

Results: The most important determinant for serum CRP and serum triglycerides levels were the visceral adipose tissue, compared with appendicular lean mass. The various levels of appendicular lean mass did not significantly add to the associations with serum CRP or serum triglycerides. However, a moderate- or high level of appendicular lean mass were associated with lower levels of HbA1c % in the groups with high levels of visceral adipose tissue. For the groups with moderate- and low levels of visceral adipose tissue there were no added associations with appendicular lean mass levels and HbA1c.

Conclusion: The results indicate that the level of visceral adipose tissue is the most important predictor for inflammatory- and metabolic biomarkers. However, the level of appendicular lean mass may be of relevance for HbA1c levels in individuals with high levels of visceral adipose tissue. The association were statistically significant, but minimal. One could therefore argue that the association is not of clinical relevance.

Abbreviations

DEXA – Dual-energy X-ray absorptiometry

MS – Metabolic syndrome

T2DM – Type 2 diabetes mellitus

CVD – Cardiovascular disease

TG - Triglycerides

BMI – Body mass index

WC – Waist circumference

WHR - Waist over hip circumference

CT – Computed tomography

MRI – Magnetic resonance imaging

HDL – High density lipoprotein

LDL – Low density lipoprotein

CRF – Cardiorespiratory fitness

SVR – Skeletal muscle mass to visceral fat area ratio

CRP – C-reactive protein

VFA – Visceral fat area

ALST – Appendicular lean soft tissue

HbA1c – Hemoglobin A1c (Glycated hemoglobin)

VAT – Visceral adipose tissue

E % - Energy percent

SD – Standard deviation

CI – Confidence intervals

ALM – Appendicular lean mass

CM – Centimeter

CM^2 – Square centimeter

KG – Kilograms

KG/M^2 – Kilograms per square meter

MG/L – Milligrams per liter

MMOL/L – Millimole per liter

RA – Rheumatoid arthritis

HighVAT – High levels of visceral adipose tissue

ModVAT – Moderate levels of visceral adipose tissue

LowVAT – Low levels of visceral adipose tissue

HighALM – High levels of appendicular lean mass

ModALM – Moderate levels of appendicular lean mass

LowALM – Low levels of appendicular lean mass

Coeff. – Coefficient

BIA – Bioelectric impedance weight

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1 Introduction

1.1 Obesity – a global health issue

Obesity has turned in to be one of the most noticeable epidemiological trends and is currently recognized as one of the severest global health issues (1, 2). A study from 2019 stated that every third person in the world is categorized as overweight or obese (3). Obesity is constituted by a body composition imbalance that affects our morbidity and mortality negatively (1, 4). The condition is linked to several chronic diseases, such as metabolic syndrome (MS), type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD) (4, 5). Long-term higher energy intake compared to total energy expenditure is one of the main reasons for obesity development (6). The reasons for the growing trend of obesity are complex. However, one important explanation is that energy dense food is becoming easily accessible and affordable around the world (4). Important factors on individual level are genetics, overeating, physical inactivity, diseases and/or medication which has weight gain as side effect (1). Additionally, impairments in social factors such as family, environment, education and economy have influence on individuals capability of managing their health and are therefore risk factors for developing obesity (6). The mentioned factors (in addition to many others) are some of the reasons for the trend of obesity we are experiencing. In Norway, we have had a reduction in several risk factors (smoking, cholesterol, and hypertension) for lifestyle diseases. The reason for this is both that the number of smokers is decreasing and that the medical treatment has improved over the recent years (4). However, the growing trend of obesity, both in Norway and the world in general, is anticipated to counter this favorable trend for risk factors of lifestyle diseases. The national institute for public health in Norway is worried what this growing trend of obesity means for the development of life style diseases, such as CVD (4). Furthermore, the proportion of young adults with obesity has increased over the last decades in Norway. The study “Fit futures 2”, conducted in Tromsø between 2012-2013, discovered that 21 % of women and 28 % of men between the age of 18-20 years were categorized as overweight or obese (4). In addition, a study from Norway which examined the development of children found that 8% of children in the third grade had visceral obesity between 2008-2012 (7). A study investigating the prevalence of obesity in the Organization for economic co-operation and development-countries (OECD-countries) (Norway is a member) found that 19.5 % of people in OECD-countries were obese in 2015

(1), and in America almost 40 % of the population was categorized as obese (1). This indicates that obesity is a problem in several generations and will remain a health issue for the coming years. In addition to the obesity trend, studies on the Norwegian population have indicated that there is a non-favorable trend in body composition as well. One longitudinal study investigated the changes in body composition based on data from the Tromsø study and found that the population had an increase in body fat, while the level of muscle mass did not change (8). Over the recent years the body composition, and especially the distribution of adipose tissue has been recognized as an important variable for predicting the risk of several lifestyle diseases (9). Obesity calculated by body mass index (BMI) assumes that the excess body fat is evenly distributed and due to heterogeneity in the location of where the fat tissue accumulates in individuals, one could argue that the BMI measure is not precise on an individual level. A more advanced and precise method is needed to estimate the body composition to highlight the importance of it in a health and disease point of view.

1.2 Methods to estimate body composition

Body mass index (BMI) and waist circumference (WC) are conventional methods used to categorize individuals based on their anthropometrics (2, 10). This is because these methods are feasible and provide a relatively good estimate on a population level (2). BMI is measured by using height and weight of an individual. The equation for BMI is $\frac{Weight}{Height^2}$, and the result is used for assessment of health risks. A BMI of more than 25 kilogram per square meter (kg/m^2) is considered as overweight and over 30 kg/m^2 is considered obese (4). Additionally, BMI may also be used to assess underweight, and a BMI under 18.5 kg/m^2 is considered underweight, and a BMI under 16.5 kg/m^2 is considered severely underweight (11). However, BMI is not able to measure the distribution body fat and muscle tissue, thus are BMI not always accurate on an individual level. A person with a high level of skeletal muscle tissue and small level of body fat may be categorized as overweight because of their weight, although they are not at risk of lifestyle diseases (9). In addition to this, BMI does not measure the distribution of body fat and muscle tissue. Therefore, a person categorized as "normal weight" may have unhealthy levels of fat tissue in exposed areas, and thus be at risk of lifestyle diseases (9). In recent years, the distribution of the fat mass has been identified equally important to predict risk for metabolic diseases as total amount of fat (12, 13). Furthermore, the abdominal fat has been identified as more problematic, and furthermore the

visceral fat has been identified as more problematic compared with subcutaneous fat in the abdominal area (14-16). WC gives an estimate of the size of adipose tissue in the abdomen area and are therefore an indicator for the risk of metabolic complications. A WC of >94cm for men and >80cm for women increases the risk and a WC of >102cm for men and >88cm for women increases the risk substantially (10). In the 1980s abdominal waist over hip circumference (WHR) were a normal method to measure fat distribution, and several studies found that WHR was predictive for increased risk of both CVD and T2DM (17, 18). Nevertheless, WHR is not able to precisely measure the level of visceral- and subcutaneous fat separately. Computed tomography (CT) and Magnetic resonance imaging (MRI) are validated as accurate methods to estimate the body composition, but it is reasonable to argue that they are not optimal in a population-based study. The reason CT is not suitable is that the radiation dose is high, in addition to expensive equipment and the need of highly qualified personnel (19). The radiation dose from the MRI is smaller, but the process is manual and time consuming. It is necessary with highly qualified personnel for the MRI and there are multiple protocols for the method, which influence the standardization when investigating muscle mass (19). In addition, the MRI is also quite expensive. When all these limitations are taken in consideration the CT and MRI are not well suited for a population-based study, however it may be feasible for a small sample study (19).

A more feasible method to measure the body composition in a population-based study is a dual-energy X-ray absorptiometry (DEXA) scan. A DEXA-scan provides a three-compartment model of the body composition by using x-ray pictures to distinguish fat-, lean-, and bone mass (19, 20). The DEXA primarily identifies bone- and fat tissue, and the remaining tissue is considered “lean mass”. Lean mass consists of a majority of muscle mass and is therefore considered a proxy marker for muscle mass. Nevertheless, lean mass also consists of other types of tissue and molecules that is not fat mass or bone mass, for example connective tissue, organs, and water (19, 20). The lean mass measured by DEXA often leads to an overestimation of muscle mass (21). This is because the torso and abdomen consist of a lot of tissue which is categorized as lean mass but are not muscle mass (19, 21). A more precise estimation of the muscle mass is to measure the appendicular lean mass (ALM). The ALM consists of lean mass from the legs and arms and exclude the lean mass from the torso and abdomen (19). When measuring ALM the estimation of muscle mass is more similar to the measurements of CT (21). In addition to estimating the muscle mass the DEXA-scan also

provides an accurate measure of the visceral fat mass. By using a recently developed software (CoreScan – EnCore version 17.0), the DEXA-scan calculate the level and volume of the visceral adipose tissue (VAT) (21). The DEXA-scan is a rare and expensive instrument which few have access to (21). Nevertheless, DEXA is not as rare as MR and CT and the patient burden is lower, with a fast, noninvasive procedure that provides a very negligible radiation dose. The lower radiation dose is an important reason for why DEXA is a more feasible method in a population-based study, compared with CT and MRI. Weaknesses of the DEXA-scan is that the availability is low, and that hydration status and excessive body fat can affect the results. Additionally, muscle mass in the trunk and torso, and individual muscles are not possible to measure. Very tall and severely obese individuals may not be appropriately placed within the scanning area and may therefore not be able to be measured (19). However, when assessing all the negatives and positives of the different methods, DEXA is the most accurate and feasible method for a population-based study.

1.3 Adipose- and muscle tissue

Adipose tissue mainly consists of fat cells (often referred to as adipocytes) and small amounts of fibroblasts, endothelial cells and immune cells (base substance) (22). The adipose tissue is located under the skin (subcutaneous fat) and around the internal organs (visceral fat). There are two types of adipose tissue, yellow/white and brown (22). The yellow/white type is of utmost importance for adults. It is important for energy storage, padding and isolation. In addition is the brown fat tissue noticeably smaller compared to the yellow fat tissue in adults (22). Adults mainly have this type of tissue in small amounts between the shoulder blades and around the kidneys (22). The brown adipose tissue is more dominant in children, and therefore more important for them. The brown adipose tissue specializes in heat production because of their large content of mitochondria (22). Drown adipose tissue is therefore important for temperature control in children.

Obesity is a condition where excessive adipose tissue accumulates and affects a person's health. The reason for the negative effect of obesity is that adipose tissue secretes a variety of adipokines (also known as cytokines), which are signaling proteins that interact with different biological processes in the body (9). Adipocytes (fat cells) secrete most of the adipokines, but when obesity occurs, immune cells also play a significant role. Macrophages are especially important, as they infiltrate the adipose tissue and secret pro-inflammatory cytokines, such as

tumor necrosis factor-α (TNF-α) (23, 24). All these mentioned consequences of obesity increase the risk of several lifestyle diseases (24). Several other mediators and hormones are also associated with the inflammation caused by obesity, for example 1β, monocyte chemo-attractant protein 1, adiponectin and leptin (24).

As mentioned, the distribution of adipose tissue has been recognized as an important predictor for the risk of disease (9). The size and location of the adipose tissue is a decisive factor for adipokine secretion and the effect of these signaling molecules (25). Visceral obesity is a term which is used to describe when excessive adipose tissue accumulates around internal organs in the abdominal cavity (9). As of now, there is no universal definition of a threshold value for the level/volume of visceral fat mass which is considered as visceral obesity. The reason for this is that existing research has different definitions of visceral obesity and have measured it differently. It is therefore hard to compare and furthermore conclude a specific threshold value based on the existing research (9). Like obesity, visceral obesity is known to increase the secretion of pro-inflammatory molecules. TNF-α, plasminogen activator inhibitor-1 and interleukin 6 (IL-6) are some of the cytokines secreted from the adipose tissue when visceral obesity occurs. These molecules are known to increase the risk for disease through altered insulin sensitivity, lipolysis, fibrinolysis and cause a low-grade inflammation (9). Individuals with visceral obesity are often associated with a dyslipidemia state. This state is associated with elevated levels of triglycerides (TG), low levels of high-density lipoprotein (HDL) and low-density lipoprotein (LDL)-levels close to normal. However, the number of LDL-particles is often higher even though serum levels appear normal. The reason for this is that the LDL-particles are smaller and denser. In short, small and dense LDL-particles have a reduced ability to bind to LDL-receptors, which is LDL-particles main route of clearance. In addition, these particles more frequently facilitate into the vascular wall where they are vulnerable for oxidation. When immune cells (macrophages) break down these LDL-particles in the vascular wall they convert in to foam cells, which leads to plaque formation, which in turn increases the risk of CVD (9). The dyslipidemia state associated with visceral obesity are therefore not favorable.

Gender and age are key factors when it comes to distribution of fat tissue. Men and women tend to store fat tissue in different areas of the body (9). Men usually stores fat in the upper body, which include the visceral fat area. While women tend to accumulate fat tissue in the

lower part of the body, for example hips and thighs (9). Because of the difference in distribution of fat tissue is the total level of body fat not as strongly correlated to the visceral fat mass amongst women, compared to men (9). As mentioned, age affects the distribution of fat tissue as well. Age affects the location of accumulation of fat especially within women. Studies have shown that post-menopausal women tend to store fat to a greater extent in the abdominal area compared to premenopausal women (9). For men the location of fat does not change with age, but studies have shown that the WHR increases with age in both men and women (9). The effect and location of excessive adipose tissue is therefore complex, and more research is needed.

Even though there are numerous convincing studies which prove the negative effects of obesity there is still one group where some studies have shown that obesity is beneficial (26, 27). This is called the "obesity paradox" and is relevant for patients with CVD. Studies have proved that individuals with a BMI up to 35 kg/m^2 have a better prognosis compared to underweight or lower end normal weight individuals (26). The mechanism behind this paradox is unknown and the paradox is most present in un-fit individuals. Fit individuals have a good prognosis, regardless of BMI and body composition (26). This is called the "fat but fit" phenomenon. The method used to measure the fitness level of individuals in these studies is cardiorespiratory fitness (CRF). Studies have shown that an obese individual with high CRF almost have the same mortality risk as normal weight individuals (27). In addition to this, individuals with low CRF had twice the mortality risk even though they were considered normal weight by BMI (27). The level of muscle mass is possibly a factor which could explain the obesity paradox and possibly contributes to the positive effect seen in "fat but fit" individuals.

There are three types of muscle tissues, skeletal-, smooth- and heart muscles (22). With the help from tendons the skeletal muscles are connected to the skeleton. The main functions of skeletal muscles are to make it possible to move joints, stabilize joints or prevent movements joints (22). The heart muscles are important for the heart to be able to squeezing blood through the circulatory system (22). The smooth muscles are mainly located in the body's hollow organs and pipe structures (22). Examples are the digestive tract, blood vessels and the bladder. The muscle tissue contributes to roughly 40-50 % of the total body weight and similar to adipose tissue, muscle tissue also secretes cytokines, often referred to as myokines

(28). The myokines affect a variety of different biological processes in the body, such as increased glucose uptake, increased lipolysis, increased thermogenesis and increased glucagon-like peptid-1 secretion from pancreas and the gut (28). Physical activity is important for secretion of myokines and for the muscles to function optimally as an organ (28). For example, long term high physical activity level has been associated with a decreasing level of stress hormones, while physical inactivity is associated with a systemic low-grade inflammation (28), similar to visceral obesity. Muscle mass has been identified as an important tissue for insulin-dependent glucose uptake, and a low skeletal muscle mass to visceral fat area ratio (SVR) has been shown to be negatively associated with MS and T2DM (29). Like adipose tissue, muscle tissue is affected by age. Both the size and strength of the muscle tissue are affected when ageing (30). However, studies have shown that with old age the muscle strength is reduced to a greater extent compared to the size of the muscles (31). Healthy and well functional muscle tissue has a lot of positive effects, and the interaction between muscle tissue and visceral obesity is a topic which needs more research.

1.4 Inflammatory- and metabolic biomarkers

C-reactive protein (CRP) is an acute phase protein which is produced in the liver. CRP is secreted by stimulation of macrophages into the blood stream during inflammation (32). CRP is therefore used to measure the degree of inflammation in the body (32, 33). Several diseases and conditions are associated with increased serum levels of CRP, for example diseases and conditions causing cell necrosis. However, some infections and inflammatory conditions which do not cause cell necrosis may cause moderate increased levels (32). There are in addition several other conditions which could affect the serum levels of CRP (34). Depression, smoking, and diabetes are associated with mildly increased CRP levels, and non-steroidal anti-inflammatory drugs (NSAIDs) are associated with false low levels of CRP (34). The increased levels are measurable in the blood stream 6-12 hours after the start of the inflammation process (32). Measure of CRP could be performed by venous blood sample or by a drop sample from the finger. The concentration is measured by milligrams per liter (mg/l), a healthy person normally has a concentration under 1 mg/l. A concentration over 5mg/l indicates an infection or inflammation (33). During an inflammation, dependent on severity, the concentration of CRP could reach more than 200mg/l (32). The half-life of CRP is approximately 20 hours, which means that the serum levels are a good measure of pace of

the production and strength of the stimulus (32). The half-life also makes it possible to assess whether the treatment is successful or not after 15-25 hours (32). As mentioned, obesity causes low-grade inflammation in the body, and increases the secretion of several inflammatory proteins, such as CRP (23, 24). However, muscle tissue has the ability to secrete anti-inflammatory myokines, which in turn possibly could influence the negative effect of obesity (35). For example the myokine IL-6 is associated with the anti-inflammatory effect seen after exercise (35). IL-6 is associated with inflammation as well, so the mechanism of action for IL-6 is complex (25). However, there are several other factors and conditions which could influence the secretion of CRP, and this should be taken into consideration when the analysis is assembled in the present thesis.

HbA1C is glycated hemoglobin and is a measure of the long-term concentration of blood glucose (36). The measure is done by a blood sample and indicates the average blood glucose through the last 6 to 12 weeks. The test does not measure the amount of free glucose, but the amount of glycosylated hemoglobin. HbA1C is the recommended method to diagnose diabetes mellitus. A concentration of 20 to 42 mmol/mol is considered normal, and a concentration over 48 mmol/mol is the limit for being diagnosed with diabetes (36). HbA1c could also be stated in percentage value. A percentage of 4 to 6 is considered normal, and concentration above 6.5 percent is the limit for being diagnosed with diabetes (37). Obesity increases the risk of T2DM and insulin resistance, which is displayed with elevated levels of HbA1C in the blood (5, 36). It is important to note that there are other factors which could affect the levels of HbA1C, for example conditions which reduce the survival rate of red blood cells could decrease the level of HbA1C (38). Acute/chronic blood loss and hemolytic anemia are examples of these types of conditions (38). On the contrary, patients with uremia have been shown to have elevated levels HbA1C even though they have a normal glucose tolerance (38). In addition, pregnancy and other types of anemias have been shown to influence the level of HbA1C (38). Therefore, it is important to take in consideration that there are other factors which could affect the HbA1C levels in the analysis of the present thesis.

“Blood lipids” is an umbrella term for the different lipids and lipoproteins in our blood that consist of cholesterol, TGs, phospholipids, and fatty acids. Measuring total cholesterol is a way to estimate the risk of cardiovascular disease. Total cholesterol measures the total

concentration of lipoproteins in the blood. The lipoproteins consist of LDL, HDL, very low-density lipoprotein (VLDL) and chylomicrons. Visceral obesity is associated with elevated levels of TG, low levels of HDL and relatively normal levels of LDL (9). However, the LDL particles are often small and dense which makes them hard to remove from the circulation. This in combination of high TG levels, and low HDL levels increases risk for CVD (9). The level of TG in the blood should not exceed 1,7mmol/L and an elevated level of TG are associated with insulin resistance, low HDL levels and increased risk for CVD (39). The reason for elevated TG levels in obese individuals is that the liver experiences an increasing flux of fatty acids, which in turn stimulates an increased production of TG (9). The association with visceral obesity makes serum TG a reasonable and feasible metabolic biomarker to measure to investigate the association between body composition and visceral obesity.

1.5 Comparable studies

As mentioned, several studies have showed that visceral obesity causes systemic low-grade inflammation and increased risk of several diseases (9, 40, 41). One study explored the possibility that muscle mass potentially could reduce risk for CVD (42). Children and adolescents were included in the study, and they examined how various levels of DEXA measured lean mass effected CVD abnormalities in individuals with different ranks of BMI. TG, LDL-cholesterol, blood pressure, total cholesterol and fasting blood glucose was some of the factors they investigated. The study found that a greater level of lean mass was protective on several of these factors, such as TG, high LDL-cholesterol, hyperglycemia and insulin resistance (42). This indicates that the level of skeletal muscle mass possibly has a protective association against inflammatory- and metabolic biomarkers associated with visceral obesity. Another study conducted in Korea investigated whether there was a correlation between the level of visceral adipose tissue and sarcopenia (age-related loss of muscle function and muscle). The study used DEXA to estimate appendicular lean soft tissue (ALST), which is a proxy measure of muscle mass, and CT to estimate visceral fat area (VFA), which is a measure of visceral fat tissue (29). 379 men and women with a mean age of 52 were included in the study. They found that there was a negative association between baseline VFA and changes in ALST (29). This indicates that visceral fat tissue may influences the level muscle mass. The study also found that women with visceral obesity at baseline had a significant

decrease in ALST compared with women without visceral obesity at baseline. Visceral obesity was defined as a VFA over 100cm^2 in men and women (29). These studies suggests that there may be an interaction between visceral fat tissue and muscle tissue. However, the Korean study investigated a very small sample (42), and the study investigating children are not generalizable for the general population. Additionally, little research investigates the mechanisms behind this interaction and how muscle mass is associated with inflammatory- and metabolic biomarkers associated with visceral obesity.

1.6 The aim of the study

The development of obesity is turning into a global health issue, and the prevalence of overweight and obese individuals has doubled over the last 35 years (3). Visceral obesity is associated with an elevated level of low-grade inflammation in the body and increasing the risk of several lifestyle diseases (9). Less is known about how muscle mass influences the proinflammatory effect of visceral obesity. This will be elucidated in this thesis by analyzing population-based data with a wide range of visceral adipose tissue- and muscle tissue levels, in addition to measured levels of inflammatory- and metabolic biomarkers. This study aims to investigate how various levels of appendicular lean mass influence the inflammatory- and metabolic biomarkers associated with visceral obesity.

2 Research question

How does appendicular lean mass influence the association between visceral obesity and inflammatory- and metabolic biomarkers?

2.1 Objectives

1. To describe different population profiles based on combinations of visceral fat mass and appendicular lean mass
2. To investigate how different combinations of low-to-high visceral fat and appendicular lean mass respectively associate with serum CRP, HbA1c and serum TG (inflammatory- and metabolic biomarkers).
3. To evaluate whether there might exist a “protective effect” of high appendicular lean mass on the association between high visceral fat mass and inflammatory- and metabolic biomarkers.

3 Method

3.1 Data collection and study sample

The data for the present master thesis was drawn from the seventh Tromsø study. The Tromsø study is a population-based cohort study conducted in the municipality of Tromsø with a total of seven surveys performed since 1974. In the last survey (7^{th}), men and women between 40-99 years old were invited to join the study between 2015-2016 (43). In total, 32,591 individuals were invited to the study, of which 21,083 (65 %) attended (43). All the participants went through the basic examinations that included a questionnaire, anthropometric measurements, and blood samples (43). Approximately 13,000 were randomly invited to extended examinations, including more advanced measures, such as a DEXA-scan that provides information on body composition and bone density (44). Of the 13,000 invited, 8,346 (64%) participated in the extended examinations. The remaining 36% did not participate in the basic study or withdrew from the study before the extended examinations. 3,683 of the 8,346 (44%) were randomized to measure the body composition with the DEXA-scan. Some of the 3,683 were also participants who measured body composition in the previous Tromsø survey (the sixth Tromsø study) (43). The age span of the participants which measured body composition was 40-84 years.

3.1.1 Inclusion- and exclusion criteria

As the present thesis aimed to investigate associations between body composition and inflammatory- and metabolic biomarkers in a population-based sample, all available participants as possible were included in the study. Instead of excluding participants with potential confounders were the confounders adjusted for in the main analysis, to maintain a representative sample from the population and secure external validity. However, some criteria were made for the participants to enter this thesis. Eligible participants for inclusion were those who provided blood samples (n=21,071) and underwent a DEXA-scan (n=3,683) in Tromsø 7. The sample included 3,661 participants after removing subjects without an adequate measure of the body composition from the DEXA-scan. 47 participants were additionally excluded after removing participants which did not have a serum CRP, serum TG or HbA1C measurement. In addition to this, participants who did not answer the self-reported disease question in the questionnaire were excluded from the analysis (n=274). These

participants were excluded because the disease variable was included as confounding factor in all the main analyses, and the number of participants with missing value were not considered high. Imputation of this variable was abandoned because the reason for why the participants did not answer the question was not known and probably complex. After removing participants which did not meet these criteria, a total of 3,340 participants were included in the main analysis. In total 1,916 women and 1,424 men. Figure 1 illustrates the inclusion/exclusion process.

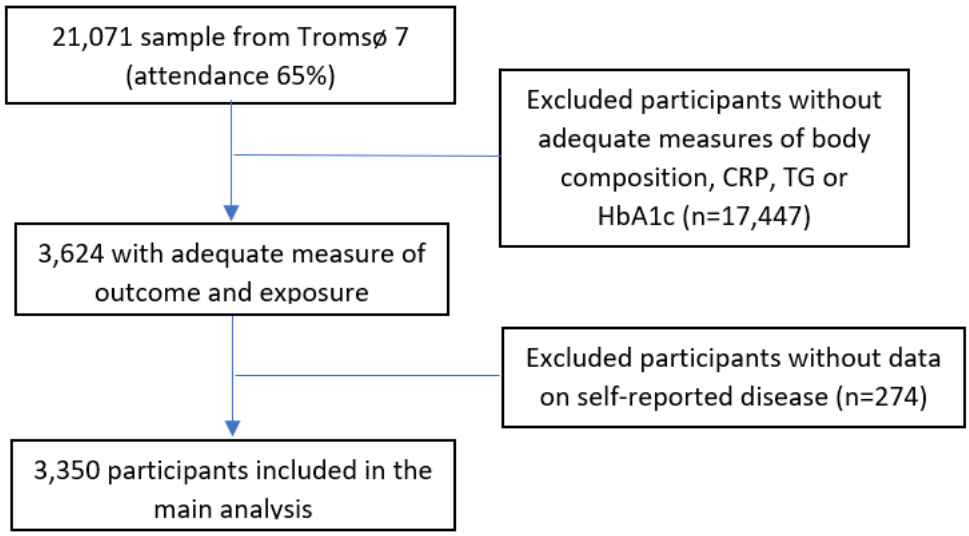

Figure 1 - Flow chart of the inclusion/exclusion process

3.2 Measures of exposure and outcome

The exposure of this thesis was ALM and VAT-mass merged in to a common categorized variable based on body composition. This were as mentioned measured by a DEXA-scan. The outcomes of this thesis were inflammatory- and metabolic biomarkers, measured by blood samples. Serum CRP, HbA1C % and serum TG were the included biomarkers.

3.2.1 Dual-energy X-ray absorptiometry

DEXA-scans were performed with a Lunar Prodigy Advance on 3,683 participants in the Tromsø 7 study. The measurements were completed according to manufacturer guidelines. The participants laid faced-up on the scanning table for approximately 10 minutes while the measurements took place. The measurements were done by trained technicians according to a

standardized protocol (45). Each morning, the devices were recalibrated to secure precise measurements. The thorax and abdomen were excluded in the measurement to estimate the ALM and only lean mass from the arms and legs were included (21). By using the software Corescan (EnCore version 17.0) the VAT-mass of the participants were estimated. Both the VAT-mass and ALM were estimated in grams by the DEXA measurement.

3.2.2 CRP, HbA1c and TG

Serum CRP, HbA1c % and serum TG were measured by blood sample collection in the seventh Tromsø study. The participants were not asked to fast beforehand, and the blood sample was therefore taken non-fasting. This measurement was part of the main survey which all participants completed. All the examinations were conducted by trained technicians using a standardized protocol. The blood samples for serum CRP, serum TG and HbA1c % were all analyzed by the department of Laboratory Medicine at the University Hospital of North Norway (ISO certification NS-EN ISO 15189:2012) (45). The blood samples were centrifuged for 10 minutes after resting in 30-60 minutes in room temperature (43). Later were they transferred to plastic tubes and kept in a room with 1 to 10 degrees (43). The measurement of serum CRP was provided in mg/L, HbA1c was provided in % and serum TG was provided in mmol/L.

3.2.3 Questionnaire

All participants in the study went through a series of questionnaires which covered several different topics. The first two questionnaires collected information about social demographic, general health, disease and medication and quality of life, with more (43). The third questionnaire covered the feeling of pain and exhaustion in the population and were called the GRIP (Graphical Index of Pain) questionnaire. After this, the participants had a physical meet up for the main examinations. On attendance for this examination, the participants received a detailed food frequency questionnaire (FFQ) which collected data regarding food and dietary habits in the population. This questionnaire obtained information which designed the dietary variables.

3.3 Statistical method

The statistical data program STATA version 17 (StataCorp, College Station, TX, USA) were used to run all the statistical analysis. To acquire descriptive statistics of the population independent two-samples t-test was used on continuous variables. Percentages were calculated and presented for the dichotomous variables. These descriptive statistics were used to investigate the differences between men and women included in the study. Means, standard deviation, number of participants and percentages were used to describe the study sample. Mean is presented with mean ± standard deviation (SD) in the results.

Multiple linear regression models were used to examine the association between the different body composition groups and metabolic- and inflammatory biomarkers. Two individual analyses were run for each of the three outcome variables, serum CRP, HbA1c % and serum TG. One analysis was run only adjusted for sex and one adjusted for all relevant confounding factors. This was done to evaluate the impact of the confounding variables. The analysis adjusted for sex only were called "model 1" and the analysis adjusted for relevant confounding factors were called "model 2". Different confounding factors were included to the serum CRP, HbA1c and serum TG in model 2. The reason for this was that different confounding factors were considered relevant for each outcome. The confounding factors for each outcome were found through Direct acyclic graphs (DAGs) and through including and excluding variables to the analyses and assessing their impact. In addition to that, collinearity tests were conducted. Collinearity tests were mainly run to assess how the dietary variables impacted each other. For example, total energy intake was not included in the analysis for serum CRP, serum TG and HbA1c % based on the collinearity tests. Age, physical activity, alcohol intake and energy percent (E %) from omega-3 (only included in the CRP and TG analysis) got a high value in the collinearity test in all the analyses. However, these variables did not affect the main exposure variables and were considered important confounding factors for the three outcomes. Age, physical activity, alcohol and E % from omega-3 were therefore included in the main analyses.

For HbA1C, the linear regression analysis calculates β-coefficients with 95 % confidence intervals (CI). P-values < 0.05 were considered significant. Serum CRP and Serum TG was not normally distributed and was therefore log transformed to better fit the linear regression

model. Appendix 1 illustrates the distribution of serum CRP and serum TG before and after log transformation. Because of this the β-coefficient for these variables is presented in an exponential form, similar to odds ratio, with 95% CI were P-values <0.05 were considered significant.

Appendix 2 contains model 1 and 2 for serum CRP, HbA1c % and serum TG with the low ALM group as reference group for the moderate- and low VAT- groups separately. This was done to investigate associations between the ALM and the groups with different levels of VAT-mass. These analyses were not included in the results because of insignificant results.

3.3.1 Variables and data transformation

Appendix 3 summarize all included variables (exposure, outcome and confounding variables), and describes their design. Both the lean mass and VAT-mass were initially continuous variables given in grams. To make it easier to interpret the effect of different body compositions both were categorized into 9 groups. By using STATA, both variables were first divided into three equally large tertiles for men and women separately. Men and women were divided separately to account for differences between the sexes. After men and women were divided in to three tertiles, the three tertiles for both sexes were combined into nine groups separately based on body composition. The subjects with the least favorable body composition were placed in group 0 (HighVAT-LowALM) and the subjects with the most favorable were placed in group 8 (LowVAT-HighALM). For example, group 0 consists of subjects who were in the highest tertile of VAT-mass and the lowest tertile of ALM. Further, there was a gradually better body composition in the groups up to group 8, which had the "most favorable" body composition. Group 8 consists of subjects in the lowest tertile of VAT-mass and highest tertile of ALM. The nine groups of men and women were then merged into a common nine groups. Men and women in group 0, got merged to a common group 0 (the same for the other 8 groups). This meant that sex and potential sex-differences in body composition was automatically adjusted for in the analysis (8). Table 1 summarizes the division of VAT-mass and ALM in to 9 groups, including their name in the models.

Table 1 - Division in groups based on body composition

Group name	VAT-mass tertile	Lean mass tertile
HighVAT-LowALM	High	Low
HighVAT-ModALM	High	Moderate
HighVAT-HighALM	High	High
ModVAT-LowALM	Moderate	Low
ModVAT-ModALM	Moderate	Moderate
ModVAT-HighALM	Moderate	High
LowVAT-LowALM	Low	Low
LowVAT-ModALM	Low	Moderate
LowVAT-HighALM	Low	High

VAT - Visceral adipose tissue, ALM - Appendicular lean mass

The self-reported disease variables, T2DM, RA and kidney disease, were initially categorized into three groups based on the questionnaire. The possible answers were “No”, “yes” and “yes, previously”. In the analysis, the three disease variables were merged to one dichotomous variable. Participants who answered “yes” to one or more of the diseases were placed in group 1, and participants who answered “no” or “yes, previously” on all the disease questions were placed in group 0. The currently sick participants were identified as the most influential group for the analysis, compared to the healthy and previously sick. The variable was therefore designed to differentiate these participants.

The questions regarding the use of medicine had three answer options in the questionnaire, “never used”, “Currently” and “previously, not now”. The questions were formulated as “do you use, or have you used …”. Three types of medication were included in this thesis, lipid lowering drugs, insulin, and diabetes tablets. Furthermore, these three variables were converted to dichotomous variables separately. Participants who answered “currently” were placed in group 1, and participants who answered “never used” or “previously, not now” were

placed in group 0. This was done because the current users were identified as the most influential group for the analysis. These three medications were not merged into one variable, like diseases, because all the medications were not recognized as relevant for the three outcomes. Just the relevant medications were included to the different analyses.

The question regarding smoking were categorized into three groups based on the possible answers in the questionnaire. “No”, “yes” and “yes, previously” were the three answer options. The question was formulated as “Do you smoke daily”. This variable was transformed into a dichotomous variable by including the participants who answered “yes” to one group, and the participants who answered “no” or “yes, previously” to the other group. This was done to differentiate current smokers, and the ones who do not currently smoke. The reason for this was that current smokers were recognized most influential for the analyses.

The intake of alcohol from the participants were based on the question “How often do you usually drink alcohol?”. The possible answers were: “never”, “monthly or less frequently”, “2-4 times a month”, “2-3 times a week” and “4 or more times a week”. This mean that this variable initially consisted of 5 groups. However, this variable was converted to a dichotomous variable for this thesis. Participants who answered they consume alcohol (alternative 2-5) were placed in group 1, and participants who answered they do not consume alcohol were placed in group 0. This distinguished participants who consumed alcohol and the ones who did not. This was considered relevant since alcohol consumption could affect both the exposure and outcome.

The variable for physical activity were categorized into 4 groups in the Tromsø study based on the possible answers of the questionnaire. “reading, watching tv/screen or other sedentary activities?”, “walking, cycling, or other forms of exercise at least 4 hours a week? (Including walking or cycling to work, Sunday walk etc.), “participation in recreational sports, heavy gardening, snow shoveling etc. at least 4 hours a week” and “participation in hard training or sports competitions, regularly several times a week?” were the answer options. For the analysis this variable was converted into a dichotomous variable. Participants who answered that they were active at least 4 hours a week was placed in group 1, while participants who answered that they were not active at least 4 hours a week (only the first answer option) was placed in group 0. This differentiates participants which had physical activity for at least 4

hours a week and participants with less than 4 hours of activity. The recommendation from the Norwegian directorate of health concerning physical activity for adults over 18 years, is to at least achieve 150-300 minutes (2,5-5 hours) of moderate physical activity per week (46). Based on these recommendations, this division of the variable was considered as satisfactory.

3.4 Ethics and data safety

The Tromsø study is conducted by the UiT Arctic University of Norway, and all seven surveys have received ethical approval by the Regional Committee for Medical and Health Research Ethics North (REK) (Application number: 282638). The participants have provided informed consent to participate in the study and have received information that they have the right to withdraw from the study whenever they want. Research projects need to be in accordance with laws and regulations to get access to the data from the Tromsø study. Therefore, an application to REK was sent and approved to obtain the data. During the process of working with the master thesis, the data was stored on a UiT OneDrive, with two-factor authentication and data classification as “confidential” using Uit-supported “Azure Information Protection software”.

4 Results

4.1 Study sample

The mean age of the men and women were similar, for men it was 65.7 ± 9.1 and for women 65.8 ± 8.9. The percentage of smokers and self-reported diseases were considered relatively equal, 12.3% of the women and 9.8% of the men were smokers and 11.9% women and 10.8% men reported disease. 22.5% and 26.5% reported drug use in women and men, respectively. The mean height and weight were higher in men compared to women, 176.5 ± 6.7 centimeter (cm) versus 163.2 ± 6.2 cm and 85.9 ± 13.1 kilograms (kg) versus 71.3 ± 13 kg, respectively. In addition to this, men had a higher average VAT-mass, 1.7 ± 0.9 kg versus 0.9 ± 0.6 kg, ALM, 26.4 ± 3.8 kg versus 18.3 ± 2.8 kg, and BMI, 27.5 ± 3.7 versus 26.8 ± 4.7, compared to women. As expected, height, weight, VAT-mass, appendicular lean mass and BMI differed noticeably between men and women. Mean HbA1C %, 5.75 ± 0.6 and 5.78 ± 0.5, and serum CRP, 2.1 mg/L ± 6 and 2.1 mg/L ± 4.7, were relatively equal between men and women, respectively. Mean serum TG were a bit higher in men compared to women, 1.56 mmol/L ± 0.9 versus 1.36 mmol/L ± 0.7. The clinical and metabolic characteristics mentioned from the population are summarized in table 2. These descriptive values were not adjusted with other variables.

Table 2 - Descriptive statistics

n	Men (n=1,424)	Women (n=1,916)
Age	65.7 ± 9.1	65.8 ± 8.9
Smoking (%)	9.8	12.3
Height (cm)	176.5 ± 6.7	163.2 ± 6.2
Weight (kg)	85.9 ± 13.1	71.3 ± 13
BMI	27.5 ± 3.7	26.8 ± 4.7
Disease (%)	10.8	11.9
Medicine (%)	26.5	22.5
VAT-mass (kg)	1.7 ± 0.9	0.9 ± 0.6
ALM (kg)	26.4 ± 3.8	18.3 ± 2.8
Serum CRP (mg/L)	2.1 ± 6.0	2.1 ± 4.7
Serum TG (mmol/L)	1.6 ± 0.9	1.4 ± 0.7
HbA1c (%)	5.8 ± 0.6	5.8 ± 0.5

Continuous variables are presented with mean and standard deviation. Dichotomous are present in percent. Diseases included - Diabetes mellitus Type 2, rheumatoid arthritis and kidney disease. Medicines included: Diabetes tablets, insulin and lipid lowering drugs.
VAT - Visceral adipose tissue, ALM - Appendicular lean mass, BMI - Body mass index, CRP - C-reactive protein, TG – Triglyceride.

381 (11 %) participants registered that they had T2DM, rheumatoid arthritis (RA) or kidney disease. In the groups with high VAT-mass, 22%, 15% and 17% of the participants registered one or more of the diseases. In the groups with moderate VAT-mass, 13%, 8% and 9 % registered one or more of the diseases. And in the groups with low VAT-mass, 9 %, 6 % and 5 % registered one or more of the diseases. For drug use, 808 (24 %) participants registered that they used lipid lowering drugs, insulin or diabetes tablets. In the groups with high VAT-mass 50 %, 39 % and 32 % used one or more of these drugs. In the groups with moderate VAT-mass 29 %, 21 % and 16% used one or more of these drugs. And in the groups with low VAT-mass 20 %, 15 % and 8 % used one or more of these drugs, respectively. Figure 2 illustrates the spread of disease and drug use in the various groups.

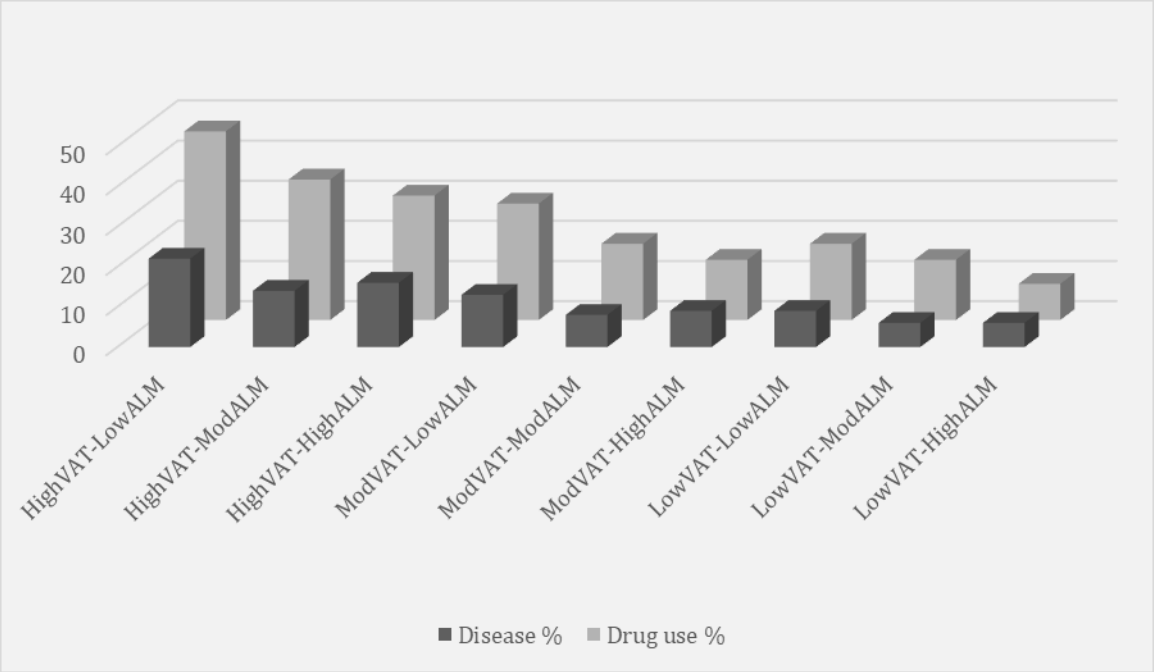

Figure 2 - Percentages of diseases and drug use in the nine groups

VAT - Visceral adipose tissue, ALM - Appendicular lean mass, Mod – Moderate

4.2 VAT-mass and ALM

All the means presented in this paragraph are listed from tertile 1-3. The mean ALM for men in the different tertiles was 22.31 kg, 26.42 kg and 30.44 kg. For women the mean ALM was 15.36 kg, 18.12 kg and 21.38 kg. The mean VAT-mass for men was 0.75 kg, 1.58 kg and 2.65 kg, and for women 0.31 kg, 0.84 kg and 1.66 kg. For men and women combined the mean ALM was 18.35 kg, 21.61 kg and 25.26 kg. The mean VAT-mass was 0.49 kg, 1.14 kg and 2.07 kg. The number of participants in tertile 1-3 was 1,117, 1,117 and 1,116 for ALM, and for VAT-mass it was 1,119, 1,117 and 1,114. Table 3 summarizes the number of participants and the mean measurement of ALM and VAT-mass for men and women combined, and separately in each tertile. This table illustrates the noticeable difference in body composition between men and women.

Table 3 - Overview of the three tertiles of ALM and VAT-mass

	Men and women combined		Men		Women	
Tertile	**ALM-Total**	**VAT-mass**	**ALM- Total**	**VAT-mass**	**ALM- total**	**VAT-mass**
Low (n)	1,117	1,119	475	476	642	643
Mean (kg)	18.35	0.49	22.33	0.74	15.40	0.30
Moderate(n)	1 117	1,116	475	474	642	643
Mean (kg)	21.61	1.10	26.28	1.56	18.16	0.83
High (n)	1 116	1,116	474	474	642	640
Mean (kg)	25.26	2.18	30.47	2.64	21.41	1.65

VAT - Visceral adipose tissue, ALM - Appendicular lean mass

The tertiles were then divided to 9 groups. The level of ALM was similar in the three Low ALM-groups, the three Moderate ALM-groups and the three High ALM-groups. In the Low ALM-groups the mean ALM was 19.00 kg, 18.27 kg and 18.16 kg. In the Moderate ALM-groups the mean ALM was 21.88 kg, 21.54 kg and 21.45 kg. In the High ALM-groups the mean ALM was 25.36 kg, 25.59 kg and 24.57 kg. As for VAT-mass the means were relatively similar in the High VAT-groups, Moderate VAT-groups and Low VAT groups separately. In the High VAT groups the mean VAT-mass were 1.96 kg, 1.99 kg and 2.16 kg. In the moderate VAT groups the mean VAT were 1.10 kg, 1.13 kg and 1.21 kg. In the Low VAT groups the mean VAT were 0.47 kg, 0.50 kg and 0.51 kg. Table 4 summarize the number of participants in the nine groups in addition to the mean ALM and VAT-mass.

Table 4 - Overview of the nine groups based on body composition

Group	Participants (n)	ALM- total (kg)	VAT- mass (kg)
HighVAT-LowALM	195	19.00	1.96
HighVAT-ModALM	345	21.88	1.99
HighVAT-HighALM	574	25.36	2.16
ModVAT-LowALM	425	18.27	1.10
ModVAT-ModALM	385	21.54	1.13
ModVAT-HighALM	307	25.59	1.21
LowVAT-LowALM	497	18.16	0.47
LowVAT-ModALM	387	21.45	0.50
LowVAT-HighALM	235	24.57	0.51

VAT - Visceral adipose tissue, ALM - Appendicular lean mass, Mod – Moderate

4.3 Main analyses

4.3.1 Serum CRP

The HighVAT-LowALM group were the reference group in model 1 and 2 for the analyses for serum CRP. In model 1 there was no significant association between the groups with the highest levels of VAT-mass and serum CRP levels, compared to the group of reference (P-value: 0.984 and 0.659). The ModVAT-LowALM, ModVAT-ModALM and ModVAT-HighALM all had significant associations (P<0.001 for all), and had 25.9 %, 32.1 % and 27.5 % lower serum CRP compared to the reference group, respectively. The LowVAT-LowALM,

LowVAT-ModALM and LowVAT-HighALM had a 52.0 %, 56.4 % and 59.1 % lower serum CRP compared with the group of reference (P<0.001 for all). In model 2, the two groups with high VAT-mass had no significant association with serum CRP levels (P-value: 0.829 and 0.455). The ModVAT-LowALM, ModVAT-ModALM and ModVAT-HighALM groups had a 26.9 %, 33.7 % and 21.2 % lower serum CRP and were all significant (P<0.05 for all). The LowVAT-LowALM, LowVAT-ModALM and LowVAT-HighALM had a 51.5 %, 52.3 % and 54.3 % lower serum CRP, compared to the reference group (P<0.001 for all). Table 5. summarize the findings from the serum CRP analysis. Model 1 and 2 with ModVAT-LowALM and LowVAT-LowALM as reference group is summarized in appendix 2.

Table 5 – Regression models for serum CRP

	Model 1			**Model 2**		
Group	Exp	Confidence interval	P-value	Exp	Confidence interval	P-value
HighVAT-LowALM	Group of reference					
HighVAT-ModALM	0.998	0.853, 1.169	0.984	0.982	0.833, 1.157	0.829
HighVAT-HighALM	1.033	0.893, 1.196	0.659	1.060	0.909, 1.237	0.455
ModVAT-LowALM	0.741	0.636, 0.862	<0.001	0.731	0.623, 0.856	<0.001
ModVAT-ModALM	0.679	0.581, 0.792	<0.001	0.663	0.563, 0.780	<0.001
ModVAT-HighALM	0.725	0.617, 0.852	<0.001	0.788	0.664, 0.935	0.006
LowVAT-LowALM	0.480	0.413, 0.557	<0.001	0.485	0.415, 0.567	<0.001
LowVAT-ModALM	0.436	0.374, 0.509	<0.001	0.477	0.404, 0.562	<0.001
LowVAT-HighALM	0.409	0.345, 0.485	<0.001	0.457	0.380, 0.548	<0.001

Model 1 - Adjusted for sex.
Model 2 - Adjusted for sex, age, smoking, physical activity, alcohol, T2DM, kidney disease, rheumatoid arthritis and energy percent from omega-3.
VAT - Visceral adipose tissue, ALM - Appendicular lean mass, Mod – moderate.

4.3.2 HbA1C %

HighVAT-LowALM was the reference group in model 1 and 2 for HbA1c%. In model 1, all the groups had significant associations with HbA1c (P<0.001 for all). The groups with the highest level of VAT-mass had lower levels of HbA1c %, with a β-coefficient of -0.177 and -0.187. The groups with moderate level of VAT-mass had a stronger decrease, with a β-

coefficient of -0.336, -0.387 and -0.437 (ModVAT-LowALM, ModVAT-ModALM and ModVAT-HighALM, respectively). The groups with low level of VAT-mass were similar to the moderate groups, with a β-coefficient of -0.386, -0.480 and -0.503 (LowVAT-LowALM, LowVAT-ModALM and LowVAT-HighALM, respectively). In model 2, all of the groups had significant associations with HbA1c. The P-values were higher in the groups with a high level of VAT-mass (P-value: 0.008 and 0.026), compared to the P-values for the rest of the groups in model 2 (P<0.001). The β-coefficient for the groups with high VAT-mass was -0.102 and -0.080. ModVAT-LowALM, ModVAT-ModALM and ModVAT-HighALM had a β-coefficient of -0.222, -0.223 and -0.208, respectively. The group with low VAT-mass had a β-coefficient of -0.246, -0.272 and -0.256 (LowVAT-LowALM, LowVAT-ModALM and LowVAT-HighALM, respectively). Table 6 summarizes the findings from the HbA1C analysis. Model 1 and 2 for HbA1c % with ModVAT-LowALM and LowVAT-LowALM are summarized in appendix 2.

Table 6 – Regression models for HbA1c %

	Model 1			**Model 2**		
Group	Coeff.	Confidence interval	P-value	Coeff.	Confidence interval	P-value
HighVAT-LowALM	Group of reference					
HighVAT-ModALM	-0.177	-0.265, -0.088	<0.001	-0.102	-0.177, -0.027	0.008
HighVAT-HighALM	-0.187	-0.269, -0.106	<0.001	-0.080	-0.150, -0.010	0.026
ModVAT-LowALM	-0.336	-0.421, -0.251	<0.001	-0.222	-0.294, -0.149	<0.001
ModVAT-ModALM	-0.387	-0.474, -0.301	<0.001	-0.223	-0.298, -0.149	<0.001
ModVAT-HighALM	-0.437	-0.527, -0.346	<0.001	-0.208	-0.286, -0.130	<0.001
LowVAT-LowALM	-0.386	-0.469, -0.302	<0.001	-0.246	-0.318, -0.175	<0.001
LowVAT-ModALM	-0.480	-0.566, -0.393	<0.001	-0.272	-0.347, -0.197	<0.001
LowVAT-HighALM	-0.503	-0.599, -0.408	<0.001	-0.256	-0.339, -0.173	<0.001

Model 1 - Adjusted for sex
Model 2 - Adjusted for sex, age, smoking, physical activity, alcohol, T2DM, diabetes tablets, insulin, kidney disease, rheumatoid arthritis and energy percent from sugar
VAT - Visceral adipose tissue, ALM - Appendicular lean mass, Mod - moderate

4.3.3 Serum TG

HighVAT-LowALM was the reference group in model 1 and 2. In model 1 the groups with high VAT-mass did not have a significant difference in serum TG (P-value: 0.776 and 0.397). The groups with moderate level of VAT-mass, all had significant associations with serum TG ($p<0.001$ for all) and had 15.8 %, 16.1 % and 17.7 % lower serum TG (ModVAT-LowALM, ModVAT-ModALM and ModVAT-HighALM, respectively). The groups with low level of VAT-mass had 37.8 %, 38.1 % and 40.9 % lower serum TG (LowVAT-LowALM, LowVAT-ModALM and LowVAT-HighALM, respectively) and all had significant associations with serum TG ($P<0.001$ for all). In model 2 the groups with high level of VAT-mass were not associated with serum TG levels (P-value: 0.728 and 0.571). The groups with moderate level of VAT-mass had 16.7 %, 18.2 % and 20.9 % lower serum TG compared to the reference group (ModVAT-LowALM, ModVAT-ModALM and ModVAT-HighALM, respectively), and all the groups had significant association with serum TG ($P<0.001$ for all). The groups with low level of VAT-mass, all had significant associations with serum TG ($P<0.001$ for all). Serum TG were 39.0 %, 39.9 % and 44.0 % lower than the reference group for LowVAT-LowALM, LowVAT-ModALM and LowVAT-HighALM, respectively. Table 9. Summarizes the findings from the serum TG analysis. Model 1 and 2 for serum TG with ModVAT-LowALM and LowVAT-LowALM are summarized in appendix 2.

Table 7 – Regression models for serum TG

	Model 1			Model 2		
Group	Exp	Confidence interval	P-value	Exp	Confidence interval	P-value
HighVAT-LowALM	Group of reference					
HighVAT-ModALM	0.991	0.919, 1.068	0.806	0.989	0.912, 1.072	0.781
HighVAT-HighALM	1.028	0.959, 1.102	0.437	1.022	0.948, 1.102	0.572
ModVAT-LowALM	0.842	0.783, 0.905	<0.001	0.833	0.770, 0.900	<0.001
ModVAT-ModALM	0.839	0.779, 0.903	<0.001	0.818	0.755, 0.886	<0.001
ModVAT-HighALM	0.823	0.763, 0.889	<0.001	0.791	0.727, 0.860	<0.001
LowVAT-LowALM	0.622	0.580, 0.668	<0.001	0.610	0.565, 0.659	<0.001
LowVAT-ModALM	0.619	0.575, 0.666	<0.001	0.601	0.554, 0.652	<0.001
LowVAT-HighALM	0.591	0.545, 0.641	<0.001	0.560	0.511, 0.613	<0.001

Model 1 - Adjusted for sex
Model 2 - Adjusted for sex, age, smoking, physical activity, alcohol, T2DM, kidney disease, rheumatoid arthritis, energy percent from sugar and energy percent from omega-3
VAT - Visceral adipose tissue, ALM - appendicular lean mass, Mod - moderate

5 Discussion

This study investigated the association between inflammatory- and metabolic biomarkers, and different combinations of ALM and VAT-mass levels in a Norwegian population. Moderate- and high levels of ALM were not associated with lower levels of serum CRP or serum TG in the groups with high VAT-mass, compared to the reference group. The most prominent association observed in the analysis for serum CRP and serum TG were between groups with various levels of VAT-mass. This indicates that VAT-mass is more important to predict serum CRP levels, compared with ALM. Moderate- and high levels of ALM were associated with significantly lower levels of HbA1c % in the groups with high VAT-mass, compared to the reference group. The association was weak, but this may indicate that the levels of ALM is an independent predictor of HbA1c % in individuals with high VAT-mass levels. However, more research is needed to support this conclusion. In the groups with moderate- and low levels of VAT-mass the levels of VAT-mass were associated with HbA1c, whereas different ALM levels were not.

5.1 The associations between body composition and inflammatory- and metabolic biomarkers

There was no indication that high- or moderate levels of ALM were associated with serum CRP for the two groups with high level of VAT-mass when compared with the reference group. In the groups with moderate- and low VAT-mass the level of ALM seems to be of little relevance, and the level of VAT-mass were associated with lower serum CRP levels. There are few studies which have investigated how ALM influence inflammation in the same manner as investigated in the present thesis, but some studies are comparable. One cohort study from Britain investigated an elderly population and found that inflammatory biomarkers, such as CRP, cortisol and Interleukin-8 (IL-8), were associated with lower grip strength, accelerated decline in grip strength, low ALM and increased risk of sarcopenia (47). The population from that study was relatively similar to the one investigated in the present thesis, with participants aged 59-70 years. In addition, they estimated muscle mass by using ALM measured by DEXA-scan, like the present thesis (47). However, the study sample consisted of approximately 300 individuals, which is noticeably fewer than in the present study. In addition, the study did not include visceral obesity or fat tissue in the analysis (47).

Obesity, and especially visceral obesity, is associated with inflammation and could possibly explain some of the associations between ALM and inflammation in the study (9). A similar study conducted in Korea investigated how visceral fat influenced skeletal muscle mass and found that visceral obesity was associated with loss of skeletal muscle in adults (29). Muscle mass were estimated with appendicular lean soft tissue (ALST). However, lower levels of ALST were not associated with an increase of VAT-mass. This suggests that VAT and inflammation biomarkers, such as CRP, are associated with loss of muscle tissue. However, the present thesis investigated if higher levels of ALM were associated with lower inflammation levels, which is the opposite pathway of the study on the Korean study investigated. Because of this it is difficult to decide if the results support the findings in the present study or not. However, the Korean study indicates that muscle mass and inflammatory biomarkers influence each other, which in a way contradicts the associations found in the present study (29). One reason for the contradicting results may be that the Korean study used a cohort study design. The cohort design makes it possible to investigate the association over time and may therefore be more appropriate to display the association between different body composition and inflammatory- and metabolic biomarkers.

This thesis is cross-sectional, meaning we only could investigate the association between body composition and the inflammatory- and metabolic biomarkers in the population at the moment the data was collected. In addition, we do not know if the exposure or outcome arrived first. One could argue that this is not the optimal method to investigate how different body compositions influence inflammatory- and metabolic biomarkers. There is however little research regarding body composition, and especially ALM, are associated with inflammatory- and metabolic biomarkers, and a cross-sectional design is a feasible to generate hypotheses and investigate if this topic needs further, and more advanced studies. A randomized controlled trial (RCT), which is considered one of the best quantitative study designs, would probably be difficult to perform based on the premises of this thesis (48). In an RCT-study the exposure is not measured before the participants are distributed into groups, to reduce risk of bias (48). However, to investigate the effect of the different body compositions it is favorable to divide participants based on their individual composition of body tissue. A feasible study design to investigate the desired issue would be a prospective cohort-study, like the Korean study mentioned in the paragraph above (29). With a cohort design, one could divide participants into groups based on the body composition, and furthermore investigate how the

body composition affects inflammatory- and metabolic biomarkers over a period of time. In addition, one could investigate the risk of disease and death. This will give an insight in how the body composition impacts the biomarkers, and possibly lead to a deeper understanding of how the different tissues influence each other. In theory it would be possible to investigate how the biomarkers change if the participants in the different body composition groups change their body composition. This is however extremely difficult in practice, because losing fat and/or putting on muscle on command are not realistic. Encouraging participants to change body composition in a possibly non-favorable manner would also raise ethical questions. The prospective cohort design is in addition time consuming and expensive to conduct (49). However, when summarizing the pros and cons are the prospective cohort is a feasible design to take the research one step further. More research with a solid and feasible study design is therefore necessary to map the total extent of association between body composition and inflammatory- and metabolic biomarkers.

The HighVAT-ModALM and HighVAT-HighALM were associated with lower levels of HbA1c % compared to the reference group. This indicates that a moderate- or high level of ALM may be positive for individuals with a high level of VAT-mass. This association is arguably not surprising because muscle mass is a large organ with a high consumption of glucose, and studies have shown that the size of muscle mass is associated with blood glucose levels (50). However, the associations found in the present study were small and one could argue that even though the associations were statistically significant it may not be of clinical relevance. The β-coefficient for the moderate- and high ALM group with high VAT-mass were -0.1. This constitutes a reduction 0.1 % in HbA1c %, for example from 5.8 to 5.7 % from the HighVAT-LowALM → HighVAT-HighALM. This would be a minimal reduction, and one could therefore argue that it is not of clinical relevance. Unlike the high VAT-groups, the association between ALM and HbA1c in the groups with moderate- and low level VAT-mass was not present.

Other studies investigating the association between body composition and HbA1c support the association found in the high VAT-groups in the present study. One study investigated the association between skeletal muscle mass to visceral fat area ratio (SVR) and T2DM and MS in adults (>18 years old) (51). The study found that SVR was associated with T2DM and MS and a non-favorable SVR increases the association with these diseases (51). HbA1c is

strongly associated with T2DM and is used to diagnose the disease, therefore one could argue that this study supports the findings from the high VAT-mass groups in the present thesis. SVR is an index which uses the ratio between skeletal muscle mass and the visceral fat area to divide individuals based on body composition. The index is not frequently used in these types of studies and one severe weakness in the study is that an inBody720 (Bioelectric impedance weight (BIA)) was used to measure both muscle mass and visceral fat area. BIA is inaccurate for measuring muscle mass and has several weaknesses for measuring body composition in general (19). The study is also cross-sectional which makes it difficult to say if the SVR or T2DM/MS appeared first. For example, T2DM/MS may cause the alteration in SVR, and not the opposite. Another cross-sectional study investigated body composition, measured by DEXA, and risk of cardiometabolic abnormalities in adolescents and children (42). They found that individuals with a greater level of lean mass had a protective association with hyperglycemia and insulin resistance (42). The population in that study is considerably different to the present thesis. Participants included were aged 6 to 18, whereas the mean age in the present thesis was 65. A noticeable weakness of that study was that it used lean mass as a proxy measure of muscle mass, which may lead to an overestimation of muscle mass (21). ALM, which was used in the present thesis, is recognized as a more accurate estimation of muscle mass compared to lean mass because ALM excludes the torso and abdomen (21). The use of lean mass may exaggerate the influence of muscle mass in this study. However, these studies in addition to several other studies found similar associations between body composition and HbA1c (or similar outcomes) which support the association from the high VAT-groups in the HbA1c analysis in the present study (50, 52, 53). This indicates that the association between body composition and HbA1c may be of clinical relevance even if the association from the present study were minimal.

The association between ALM and serum TG in the two groups with high levels of VAT-mass was not significant. There was a significant drop in serum TG from the groups with high- to moderate VAT-mass, and from high- to low VAT-mass. However, the association between ALM and serum TG within these individual VAT-groups was not significant, these results are presented in appendix 2. This indicates that VAT-mass is a stronger determinant of serum TG levels compared to ALM. There is not an abundance of research investigating the association between body composition, including muscle mass, and serum TG, but there are some. One study, mentioned in the paragraph above, initially investigated the association

between SVR and T2DM and MS, and found that SVR was inversely associated with TG concentration in the participants included in the study (51). Another study mentioned in the paragraph above investigated the association between lean mass and cardiometabolic risk in children and adolescents (42). This study found that a greater lean mass had a protective association on high total cholesterol, high LDL, hyperglycemia and insulin resistance (42). This indicates that muscle mass may be protective against several factors associated with dyslipidemia. However, there was no protective association between lean mass and TG. Both studies had methodological limitations, and methods which were different from the present thesis (these are mentioned in the paragraph above). Nevertheless, these studies indicate contradicting results regarding the association between TG and body composition and prove that there may be an association between muscle mass and TG (and/or other factors associated with dyslipidemia). Unlike muscle mass, the association between obesity and TG are well documented, and an overview study on the topic emphasized that there is an association between visceral obesity and TG levels (9). Because of the contradictory results regarding the association between muscle mass and TG more research is needed to uncover the true association. The reason for why the association between ALM and inflammatory- and metabolic biomarkers associated with visceral obesity were weak in the present thesis is most likely complex. Methodological problems may be one of them.

5.2 Methodological considerations

Inaccurate estimation of muscle mass may be one reason for why the association between ALM and the biomarkers were weak in the present thesis and that similar studies find contradicting results. Research conducted on the accuracy of estimated muscle mass from DEXA discovered inconsistent results (54). The reason is that the DEXA is not able to measure the muscle mass alone. The DEXA only divides fat and bone mineral content directly, and the rest of the tissue is categorized as lean mass (54). Lean mass is a proxy marker for muscle mass. However, the lean mass consists of muscle mass, water, organ weight etc., and may therefore give inconsistent estimations of the muscle mass (54). Inconsistent estimations of muscle mass may lead to incorrect interpretation of the association between muscle mass, health, and disease (54). Accurate estimations of muscle mass are crucial to identify the true impact of this tissue on health and disease. The same study that reported inconsistent estimations from DEXA suggests that D3-creatine dilution is more

accurate at estimating muscle mass, compared with DEXA (54). The study reported that there was a weak association between lean mass and functional capacity and risk of injuries from fall in elderly men, while muscle mass estimated by D3-creatine dilution gave a stronger association for the same outcomes (54). D3-creatine dilution is a feasible method to use population-based study as the patient burden is low, and the measuring process is relatively easy and non-invasive (54). D-3 dilution also contains fewer sources of error compared with DEXA (54). For the measurement, a dose of D-3 creatine is ingested orally. The D-3 creatine then mixes with the natural creatine pool in the body. Approximately 98 % of creatine in the body is located in the muscles, and most of the D-3 creatine is transported here. Some may be excreted in the urine before reaching the muscles, but this can be accounted for by an algorithm. The secretion of creatinine and D-3 determine the size of the creatine pool, and furthermore makes it possible to estimate skeletal muscle mass levels (54). In future studies it would be interesting to see if the D3-creatine dilution measuring technique could improve the association between muscle mass and inflammatory- and metabolic biomarkers regardless of VAT-mass.

Another possible explanation for why we did not see a strong association between ALM and the biomarkers is that visceral fat is a stronger determinant of the inflammatory- and metabolic biomarkers. An overview of the pathophysiology of visceral obesity found that there is convincing evidence that visceral obesity is an important risk factor for several health outcomes, including the biomarkers included in this thesis (9). Despite the severity of the problem there is still no accepted definition of a universal threshold-value to define visceral obesity. This makes it complicated to compare different existing research results. This may additionally have caused a non-optimal division of participants based on VAT-mass in the present thesis. In this thesis participants were divided in tertiles based on VAT-mass. Tertiles is the most objective method to divide the participants, however this may cause some groups to “miss” the possible threshold value. For example, if the visceral fat level of the study sample is generally lower than what should be considered as visceral obesity, participants may be categorized in a non-favorable manner and impair the association. A study using the same study sample as the present study found that the threshold value for the risk of developing metabolic syndrome were approximately 1.9 kg for men and 1.1 kg for women (55). The mean VAT-mass for men and women in the “high” tertile in the present study were 2.64 kg and 1.65 kg, respectively, which is well above the threshold value. The mean VAT-

mass for men (1.56 kg) and women (0.83 kg) in the “moderate” tertile were slightly under the threshold value, and the “low” tertile were significantly lower. This indicates that the negative effects associated with visceral obesity were present in the “high” VAT-groups, but that the negative effects possibly were not present in the majority of the participants in the moderate and low tertile. This may have impaired the association between ALM and inflammatory- and metabolic biomarkers. In other words, if the negative associations associated with visceral obesity are not present in these groups it is difficult for ALM to be associated with them. However, it is important to notice that TG were the only biomarker included in that study (CRP and HbA1c were not) (55). The threshold value for CRP and HbA1c are possibly different. However, a study investigating what a threshold value for the negative associations associated with visceral obesity is a step in the right direction, and more research is needed to conclude with a threshold-value. With a universal threshold-value is it possible to divide participants depending in their score for visceral obesity into two groups and investigate how different levels of ALM are associated with the biomarkers. Hopefully, this will be possible in future studies.

Linear regressions were used to investigate the association between body composition and serum CRP, HbA1c % and serum TG. The independent variable, body composition, were categorized for the analyses. This approach could be criticized because some information about the participants is shelved, and there are possibly some differences within the constructed categories could be lost. However, one of the objectives of the thesis were to investigate different body composition profiles in the population, and the categorization makes it easier to evaluate the association of different body composition profiles in the population. Categorization may make it easier to identify a threshold value for VAT-mass and ALM which is associated with unhealthy levels of the biomarkers. This is in the interest of people in general, and especially clinicians. For people in general it is interesting to know if they have “too much” or “too little” of a tissue. This is interesting for clinicians as well, and furthermore it would help clinicians to give better advice to patients and improve treatment. The tertile division also reveals the gradual difference between the different levels of VAT- and ALM, which makes it easier to investigate what difference a certain increase/decrease in these tissues constitute. If the body composition variables were continuous, we would only be able to see what one unit increase in VAT- or ALM constituted to the biomarkers. This would not be optimal to answer the present thesis objectives.

All the main analyses were adjusted for sex, age, smoking, alcohol, physical activity, T2DM, RA and kidney disease. These variables were adjusted for because they were considered as important confounders, and therefore crucial to unveil the association between body composition and inflammatory- and metabolic biomarkers. Alcohol intake and physical activity received relatively high values from the collinearity tests. However, there was no sign of collinearity between them and the body composition variable, therefore both were adjusted for in the analyses. Serum CRP and serum TG were adjusted for omega-3 as well. Omega-3 received a high value for collinearity, but there was no sign of collinearity with the body composition variable. In addition, omega-3 possesses several effects on metabolic processes and adipose tissue biology, in addition to anti-inflammatory effects and a positive effect on insulin sensitivity (56). Studies has in addition proved that omega-3 have a TG lowering effect (57). Because of these effects on both the outcome and exposure were omega-3 adjusted for in the serum CRP and serum TG analyses. HbA1c were adjusted for insulin and diabetes tablets as they influence HbA1c levels (58), whereas serum TG were adjusted for lipid lowering drugs because of their influence on lipids in the blood (59).

Alcohol consumption is associated with increased risk of several diseases (60). In the present thesis the variable for alcohol was categorized into two groups, based on if the participants consumed alcohol or not. Participants who answered that they consume alcohol monthly or less frequently were categorized as “consumers”. Some participants in this group may have a minimal intake of alcohol, and a small intake of alcohol is not necessarily associated with negative outcomes (60, 61). However, the intake of the participants may be high when they consume alcohol, even though they rarely drink. To not over complicate the alcohol variable a dichotomous division of this variable were chosen. However, a more precise measure of alcohol consumption would have been beneficial.

The data regarding use of medication, diseases, physical activity and nutritional variables were all collected by self-reporting through questionnaires. One study investigated how the self-reported utilization of healthcare agreed with the actual utilization (62). They found that the percentage of agreement ranged from 30-99 %, and that the accuracy varied noticeably between different demographic groups. This indicates that there may be bias within the self-reporting variables in the seventh Tromsø study. For example, the questions regarding medications and diseases demand that the participants have knowledge about their diagnoses

and medication, and lack of knowledge may lead to biased answers. One study conducted on a Finnish population found that self-reporting was more accurate for predicting prevalence, compared to incidence (63). For diabetes, the accuracy for prevalence was 96 % and for rheumatoid arthritis the accuracy was 83 % (63). The study used national health registers as validity criterion (63). Because the seventh Tromsø study measured prevalence does this support the accuracy of self-reported diseases, but there is a risk of inaccurate measures and bias on an individual level. In addition, self-reported food intake and physical activity from the seventh Tromsø study depend on the recall from the participants, which may cause some bias as well. However, both the food questionnaire and the questionnaire for physical activity were validated in the seventh Tromsø study (64, 65), which is a strength.

Selection bias may be present in the Tromsø study. The study included participants from the age of 40-99, and 65 % of the invited participated. The reason for why 35 % chose to not participate is unknown, but one reasonable reason may be because of disability and poor health status. A sensitivity analysis of the participants who chose not to participate in the seventh Tromsø study would have been interesting to see in the future. The internal validity in the present master thesis could arguably been better. The possible inaccurate estimation of muscle mass, potential miss of a threshold value for visceral obesity and potential bias in self-reporting and questionnaire are some of the factors which weakens the internal validity. However, the measures of the exposure and outcome are very objective, which mean that the reliability of the study is good.

5.3 Body composition profiles in the population

The mean ALM in the population was 22.4 kg (28.4 % of total body weight) in this thesis. The mean was 26.4 kg in men, and 18.3 in women. This is relatively similar to estimations made in comparable studies. For example, in a study conducted in Korea, the mean ALM was 22.2 kg at baseline, based on measurements made by DEXA (29). In addition, men had a mean ALM of 26.2 kg and women 18.1 kg at baseline, which is almost identical to this study. The mean age in that study was 52 (29). Another study based on a population in Austria measured the ALM with DEXA but estimated the ALM with kg/m^2. This is done by adding height in the equation. The mean ALM was 7.5 kg/m^2 in that study (66). If we do the same calculation on the study sample in the present thesis the mean ALM was 7.8 kg/m^2. This is also almost identical. The participants included in that study were between 18-81 years old

(66). This indicates that the ALM estimated in this thesis is relatively equal to the levels in similar studies using DEXA. The mean VAT-mass in the population in the present thesis were 1.3 kg, and 1.7 kg for men and 0.9 for women. The study from Korea did not measure VAT-mass the same way as the present thesis, but based visceral obesity on cm^2, which is hard to compare with the VAT-measurements in the present thesis but exemplifies the issues that there are no universal threshold-values (29). However, the study from Austria measured VAT-mass, and the mean were 0.9 kg. This is a noticeable difference from the population in the present thesis. The reason may be that the Austrian study included younger participants. The reason why this is an issue is that both men and women tend to store more fat in the abdomen area with increasing age, especially post-menopause women (9). If we investigate the VAT-mass for participants in the age 60-70 from the Austrian study the mean VAT-mass were approximately 1.5 kg. This group is arguably more comparable as the mean age in the present thesis is 65.8. The mean VAT-mass for participants between 60-70 were almost equal to the present thesis. These comparisons indicate that the level of VAT-mass varies noticeably with age, and that the levels of VAT-mass and appendicular lean mass in the population of the present thesis is generalizable.

The "obesity paradox" and the "fat but fit" phenomenon have been discussed in several studies over the last years (26, 27). The obesity paradox implies that individuals categorized as overweight or mildly obese have a better prognosis for CVD compared to underweight and individuals in the lower end of the "normal weight" category of BMI. One could not say that the obesity paradox is explained by the ALM of the participants in the present thesis. However, the association indicates a small improvement in the predictor for CVD diseases, HbA1c, for individuals with a high level of VAT-mass, which most likely is the heaviest individuals in the present thesis (67). Whereas we see no association with ALM and HbA1c in the individuals with low level of VAT-mass, which is arguably the lightest individuals. This is arguably not comparable, but it would be interesting to see future studies investigating if muscle mass may have a protective association with CVD in heavier individuals.

The physical activity level of the participants in this thesis is based on questionnaire data, and the variable is dichotomous (over or under 4 hours activity a week). This is according to the recommendations from the Norwegian Directorate of Health (46). However, this may not assess the fitness level of the participants satisfactory. Cardiorespiratory fitness (CRF) is a

method to measure the fitness level of an individual (27). CRF is associated with the risk of disease in individuals and seems to explain the “fat but fit” phenomenon (27). For example, one study found that obese individuals with a high CRF have similar mortality risks as individuals which were normal weight, and individuals with a low CRF had increased risk of mortality regardless of their BMI (27). Even though BMI is not an accurate measure on an individual level, this indicate that accurate measures of the fitness level of participants could be of significant importance when investigating the association between body composition and inflammatory- and metabolic biomarkers. So far there are no specific recommendations regarding resistance or endurance training for prevention of overweight or obesity in Norway (68). There are only modest recommendations considering physical activity in general for prevention of obesity. “Increased physical activity” and the general recommendations for the population regarding physical activity are recommended by the Norwegian directorate of Health for prevention (68). For the present study, the participants which is considered “fit” are the participants with high level of ALM. However, the level of ALM does not seem to explain the “fat but fit” phenomenon in the present study. In future studies it would be interesting to see if the physical activity level measured by CRF correlates with inflammatory- and metabolic biomarkers, and if the CRF is associated with the level of muscle mass.

6 Conclusion

The results from this master thesis indicates that there was no protective association between moderate- or high ALM and high serum CRP- and serum TG levels compared to individuals with low ALM levels. VAT-mass has the strongest association with both serum CRP and serum TG levels because of the noticeable difference between groups with high, moderate and low s of VAT-mass. Moderate- and high levels of ALM were associated with lower levels of HbA1c in the groups with high VAT-mass. The association was minimal, but this indicates that the ALM may have a clinical protective association on the HbA1c % levels in individuals with high levels of VAT, however more research is needed to confirm this. In the groups with moderate- and low levels of VAT-mass, the ALM have a negligibly association with HbA1c, and VAT-mass is the strongest determinant within these groups.

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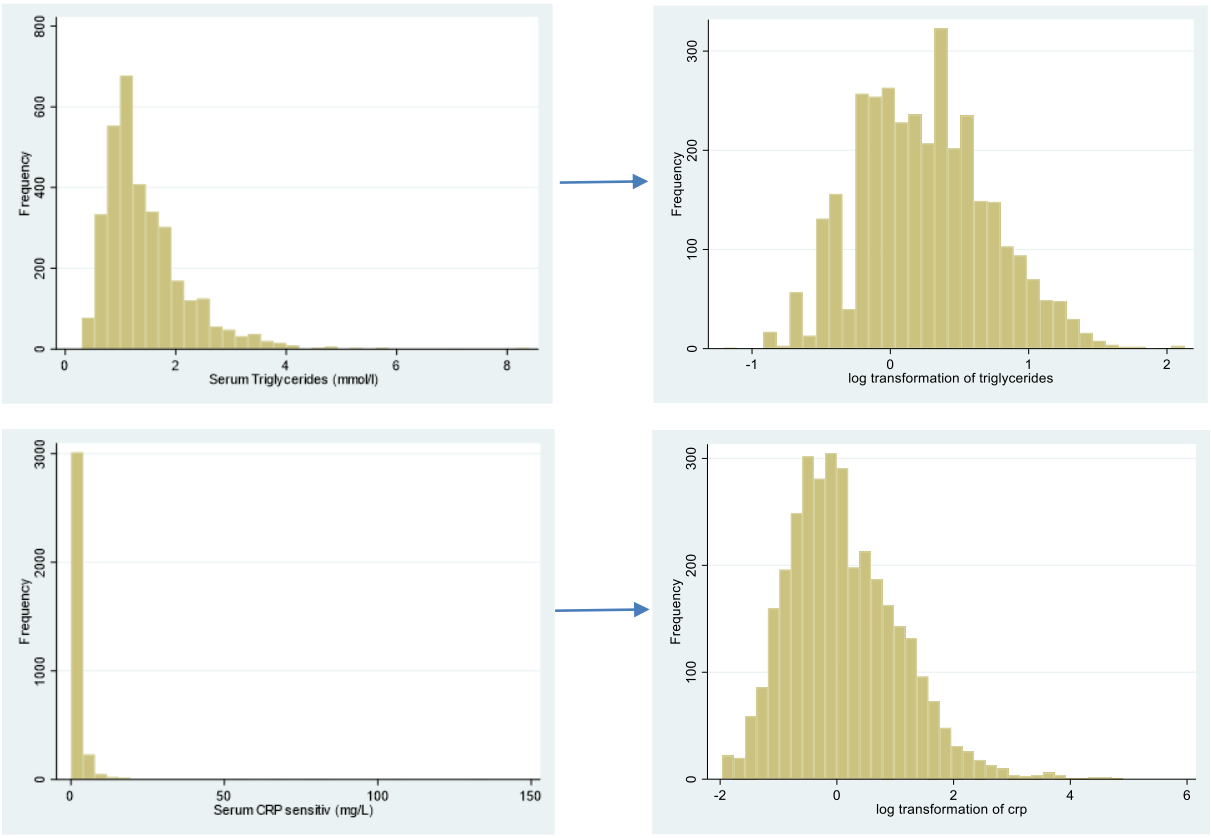
Appendixes

Appendix 1 - Distribution of serum CRP and serum triglycerides before and after log transformation



Appendix 2 - Model 1 and 2 for serum CRP, HbA1c % and serum triglycerides with different reference groups

Model 1 and 2 for Serum CRP

	Model 1			**Model 2**		
Group	Exp	Confidence interval	P-value	Exp	Confidence interval	P-value
ModVAT-LowALM	Reference group			Reference group		
ModVAT-ModALM	0.917	0.806, 1.042	0.183	0.903	0.789, 1.033	0.136
ModVAT-HighALM	0.980	0.854, 1.123	0.768	1.052	0.905, 1.221	0.511
LowVAT-LowALM	Reference group			Reference group		
LowVAT-ModALM	0.910	0.811, 1.020	0.106	1.008	0.893, 1.138	0.902
LowVAT-HighALM	0.853	0.747, 0.976	0.020	0.980	0.848, 1.134	0.787

Model 1 and 2 for HbA1c %

	Model 1			**Model 2**		
Group	Coeff.	Confidence interval	P-value	Coeff.	Confidence interval	P-value
ModVAT-LowALM	Reference group			Reference group		
ModVAT-ModALM	-0.051	-0.107, 0.004	0.069	-0.010	-0.059, 0.039	0.696
ModVAT-HighALM	-0.101	-0.160, -0.042	0.001	0.007	-0.048, 0.061	0.814
LowVAT-LowALM	Reference group			Reference group		
LowVAT-ModALM	-0.094	-0.153, -0.036	0.002	-0.023	-0.072, 0.027	0.367
LowVAT-HighALM	-0.118	-0.186, -0.049	0.001	-0.007	-0.066, 0.053	0.822

Model 1 and 2 for serum triglycerides

	Model 1			**Model 2**		
Group	Exp	Confidence interval	P-value	Exp	Confidence interval	P-value
ModVAT-LowALM	Reference group			Reference group		
ModVAT-ModALM	0.996	0.938, 1.058	0.903	0.987	0.925, 1.053	0.685
ModVAT-HighALM	0.978	0.917, 1.042	0.489	0.945	0.880, 1.015	0.122
LowVAT-LowALM	Reference group			Reference group		
LowVAT-ModALM	0.995	0.944, 1.049	0.861	1.007	0.950, 1.068	0.813
LowVAT-HighALM	0.950	0.894, 1.011	0.105	0.950	0.886, 1.019	0.153

Appendix 3 - Summary of variables included in the analysis

Variables	Description	Level of measurement
Sex	Male/female	Dichotomous
Age	Number	Continuous
BMI	Weight/height^2	Continuous
Smoking	Current smoker or not*	Dichotomous
Physical activity	Over 4 hours of physical activity per week = 1 Under 4 hours of physical activity per week = 0	Dichotomous
Alcohol	Do you drink alcohol or not*	Dichotomous
VAT-mass	The weight of the visceral fat mass in grams	Continuous
Lean mass	The weight of the skeletal muscle mass in arms and legs in grams	Continuous
Diabetes tablets	Self-reported current use of diabetes tablets*	Dichotomous
Insulin	Self-reported current use of insulin*	Dichotomous
Lipid lowering drugs	Self-reported current use of lipid lowering drugs*	Dichotomous
Serum-CRP	mg/L	Continuous
Serum-triglycerides	mmol/L	Continuous
Serum-HbA1C	%	Continuous
Type 2 Diabetes Mellitus	Self-reported Type 2 Diabetes Mellitus*	Dichotomous
Rheumatoid Arthritis	Self-reported Rheumatoid Arthritis*	Dichotomous
Kidney Disease	Self-reported Kidney Disease*	Dichotomous
Energy intake	In kilo calories	Continuous
Sugar intake (E%)	Energy percent of total kilo calorie intake	Continuous
Omega-3 intake (E%)	Energy percent from omega-3 of total kilo calorie intake	Continuous
Cis-polyunsaturated fat intake (E%)	Energy percent from cis-polyunsaturated fat intake of total kilo calorie intake	Continuous
Saturated fat intake (E%)	Energy percent from Saturated fat intake of total kilo calorie intake	Continuous

**: dichotomous variables categorized as "yes" or "no"*