The relation between serum leptin concentrations and cardiovascular risk factors in extremely obese men and women

*With focus on lipids, inflammation and insulin resistance*

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*Master Thesis*
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Abstract

Background. Obesity is associated with cardiovascular disease (CVD). However, while extremely obese patients may carry a very high risk of CVD, some of these patients are noted to have normal levels of CVD risk factors. Pathways leading to CVD include dyslipidaemia, inflammation and insulin resistance (IR). Leptin is a hormone produced by adipose tissue that is involved in these pathways. The aim of this thesis was to evaluate the independent associations between leptin and CVD risk pathways in extremely obese men and women.

Subjects and methods. In a cross-sectional study conducted at Oslo University Hospital, between 2005-10, 315 patients (62% women) aged 18-65 years, with body mass index (BMI) ≥ 40 kg/m² were recruited. Lipid profile (total-, high-density lipoprotein cholesterol [HDL C], and low-density lipoprotein cholesterol [LDL C] and triglycerides), fasting- and 2-h post challenge glucose, fasting insulin, inflammatory markers, including C-reactive protein (CRP) and ferritin, and fasting- and 2-h serum leptin were analysed. The Homeostasis Model Assessment 2 (HOMA2) indexes for insulin sensitivity (HOMA-S), insulin resistance (HOMA-IR) and β-cell function (HOMA-β) were calculated. Parametric tests were applied as appropriate. Correlations between serum leptin concentrations and selected parameters were assessed, and linear regression models were applied to investigate associations adjusting for gender and adiposity.

Results. Fasting serum leptin concentrations were higher in females compared with males (64.0 ± 33.1 ng/mL vs. 45.5±29.6 ng/mL), and fasting and post-glucose challenge serum leptin concentrations were strongly correlated to each other (R = 0.93, p < 0.001). Fasting serum leptin was significantly positively associated with BMI and body fat percentage, and negatively with hip circumference and waist-to-hip ratio. Fasting serum leptin tended to be positively correlated with HDL C in females (R = 0.14, p = 0.067) and was positively correlated with HDL C in males (R = 0.21, P = 0.040). Fasting serum leptin was inversely associated with ferritin concentrations (females: R = −0.18, p = 0.03; males: R = −0.20, p < 0.001), and positively associated with CRP in females (R = 0.24, p = 0.001). A correlation between fasting serum leptin and HOMA-IR was observed only in males (R = 0.22, p = 0.030). The associations between HDL C, CRP and ferritin and fasting serum leptin persisted after controlling for gender and adiposity (BMI, waist-to-hip ratio or body fat percentage). Including all variables in the model did not remove the significance of any of the associations.
In all analyses using post-glucose challenge serum leptin concentrations results were similar (data not shown).

**Conclusion.** The positive association between leptin and peripheral adiposity and HDL C propose that leptin is not strictly associated with increased cardiovascular risk in extremely obese individuals. Our results suggest that, in this group of extremely obese patients, leptin was independently and positively associated with CRP but negatively associated with ferritin. These findings indicate that leptin is involved in some but not all inflammatory pathways, supporting the hypothesis of selective leptin resistance (LR). The clinical relevance of our findings is uncertain. We cannot eliminate the possibility that leptin may confer both cardioprotective and pathological activities in extremely obese individuals. These data suggest that pathways leading to CVD may require separate study in extremely obese populations.
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<th>Description</th>
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<tbody>
<tr>
<td>AT</td>
<td>Adipose tissue</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index (kg/m²)</td>
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<tr>
<td>Body fat %</td>
<td>Body fat percentage</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>HDL C</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>Homeostatic Model of Assessment-β cell function</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostatic Model of Assessment-Insulin Resistance</td>
</tr>
<tr>
<td>HOMA-S</td>
<td>Homeostatic Model of Assessment-Insulin Sensitivity</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>LDL C</td>
<td>Low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>LR</td>
<td>Leptin resistance</td>
</tr>
<tr>
<td>MC4r</td>
<td>Melanocortin receptor 4</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>OB-R</td>
<td>Leptin receptor</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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1. Introduction

1.1. Obesity

Excess body weight is among the leading health concerns of the World Health Organization (WHO) European Union in the 21st century, and a large proportion of today’s global burden of disease can be attributed to obesity\(^1\). Over the past decades, the prevalence of overweight and obesity has increased at an alarming rate in both industrialized and developing countries across the world. Obesity has by now bypassed hunger on the ranking of the World’s foremost health problems, and the trend is projected to continue\(^2-5\). Epidemiological studies indicate that excess adiposity is an important risk factor for other chronic diseases, including type 2 diabetes mellitus, cardiovascular disease (CVD), cancer, and early mortality\(^6,7\). As obesity-related morbidity is increasing in parallel with the average longevity in the human population, the average number of sick-years per habitant will increase\(^8\). This will require substantial capacity in health care and place an enormous economic burden on societies’ economies. It is crucial that efforts are made to learn more about predisposing and causal factors involved in the obesity epidemic and the associated risks, so effective prevention strategies and treatment methods can be developed and implemented.

Body mass index (BMI) is an index of weight-for-height, frequently used to classify nutritional status in clinical or public health practice. BMI is defined as a person’s weight in kilograms, divided by the square of height in metres (kg/m\(^2\)). WHO has proposed an International Classification of adult weight status, based on BMI intervals (table 1). BMI is independent of age, gender and ethnicity, and does not take body composition into consideration. By definition, obesity is a condition of excessive adipose tissue (AT). However, because of the inconvenience in measuring body fat percentage (%) directly in a large number of individuals, BMI is the parameter of choice in determining the weight status of larger groups and populations. Thus BMI is not sufficient to determine adiposity in all individuals, but will give a reasonably good indication of the prevalence of obesity on the population level\(^9\). Although the risk functions of obesity are similar for most populations\(^10\), the health risks associated with different BMI intervals may vary between groups and populations, influenced by various factors such as age, gender, ethnicity, physical activity levels etc\(^9\). This should be taken into consideration when comparing different populations.
1.1.1. Prevalence

Estimations of global BMI trends, based on data including 9.1 billion participants from 960 countries worldwide, suggest that in 2008, 16% of adults globally had a BMI $\geq 25 \text{ kg/m}^2$, of which 3.3% of women and 2.3% of men were classified as obese\(^3\). Similar trends were observed in the prevalence of childhood overweight and obesity\(^5, 11\). In 2010, the global prevalence of overweight and obese children was estimated at 6.7%, and projected to rise to approximately 9.1% in 2020\(^5, 11\).

A review of relevant studies published between 1990-2008, reported that the prevalence of obesity in Europe ranges from 4.0-28.3% for men, and from 6.2-36.5% for women, with prevalence rates in Central, Southern and Eastern Europe being higher compared to Northern and Western Europe\(^12\). According to the WHO report from 2007, the prevalence of obesity in the European Union countries ranged from 5.4-22.8%, while the prevalence of overweight ranged from 31.9-79.3% in men and 27.8-77.8% in women, with highest rates reported in Albania, Bosnia and Herzegovina and in the United Kingdom (Scotland)\(^13\). The report suggests that the prevalence of obesity in the European Union countries has risen at least threefold since the 1980s\(^13\).

The 2010 report by the Norwegian Health Department\(^8\) confirms that the prevalence of obesity is on a rise also in Norway, although rates are still lower compared to other European countries. Self-reported numbers form the Norwegian adult population, show that while the share of obese adults increased only from 4 to 6% between 1973 and 1998, it increased more than twice as fast during the next decade, to 10% in 2008\(^8\). It should be noted however, that these findings are based on self-reported height and weight, and show a significantly lower

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Table 1. The World Health Organization classification\(^1\) of nutritional status

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.50</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.50 – 24.99</td>
</tr>
<tr>
<td>Overweight</td>
<td>$\geq25.00$</td>
</tr>
<tr>
<td>Obese</td>
<td>$\geq30.00$</td>
</tr>
<tr>
<td>Obesity class I</td>
<td>30.00 – 34.99</td>
</tr>
<tr>
<td>Obesity class II (morbid obesity)</td>
<td>35.00 – 39.99</td>
</tr>
<tr>
<td>Obesity class III (extreme obesity)</td>
<td>$&gt;39.99$</td>
</tr>
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</table>

\(^1\)Adapted from WHO website, 2011.
prevalence than do objective studies. The report by the Norwegian Health Department, examining studies conducted after 2000, suggested that approximately 20% of men and 17% of women in the ages 40-44 years were obese. The prevalence is increasing also in Norwegian youth and children in all ages. A recent study by Júlíusson and colleagues (2010), including 6386 Norwegian children aged 2-19 years, suggest that according to cut-off values developed by the International Obesity Task Force, 13.8% of Norwegian children are overweight or obese.

1.1.2. Extreme obesity

Extreme obesity has been defined as a BMI ≥ 40 kg/m². The prevalence of extreme obesity is on an rise worldwide, and in developed countries in particular. According to recent self-reported data from the United States, the prevalence of extreme obesity is increasing twice as fast as obesity in general, and the increase is similar in both genders and in all ethnicities, age groups and educations levels. We could not find numbers on incidence and prevalence of extreme obesity in the Norwegian population.

The health risk associated with increasing BMI increases in a non-linear manner, thus the increase is steeper with greater degrees of obesity. Extreme obesity is associated with a substantial worsening in morbidity and mortality from chronic illnesses and with poorer health-related quality of life. Cardiovascular risk, co-morbidities and mortality associated with increasing adiposity will be discussed in section 1.2.

1.1.3. Causal factors

Physiologically, obesity results from the accumulation of fat in white AT, caused by an energy intake that exceeds energy expenditure. Weight gain occurs when this imbalance, even if only modest, persists over time.

Eating is not only a method to gain energy, but also serves an important social and emotional function. Eating, and the amount of food we eat, is affected by hunger (the physiological need for energy), appetite (the desire for specific foods) and satiety. The amount of energy one person requires for maintaining energy balance depends on a number of factors, including age, gender, weight, body composition, diet and energy expenditure, the latter being a function of resting metabolic rate, diet-induced thermogenesis and physical activity levels. A
number of factors play a role in the regulation of food intake and failure of energy homeostasis. Individuals in most populations have sufficient knowledge of healthy diet and the benefit of physical activity, and yet the global population is gaining weight. A complex interplay between genetic, biologic, social and environmental factors probably affects body weight regulation. As yet, the contribution and importance of various factors have not been clarified. More than 20 genes, including the gene encoding for leptin, have been linked to body weight regulation, however genetic predisposition alone is rarely the cause of dysregulation. The role of leptin will be discussed in section 1.3.1.

Complex, redundant neuronal and humoral systems are involved in the regulation of feeding and expenditure, reflecting the fundamental biological necessity of adequate nutrient supply and energy homeostasis. Various neuronal and endocrine pathways play a part in the process of conducting information about energy and nutrient status to the brain, which, in turn, exerts the appropriate autonomic, endocrine and behavioural output. The hypothalamus and the caudal brain stem are critical sites in the regulation of energy homeostasis. For example, destruction of various sites, including the ventromedial, paraventricular and dorsomedial nuclei, induces hyperphagia. Areas in the cortex and limbic system are involved in integration of information of reward, emotion and social and environmental factors.

The human body requires sufficient amounts of energy and nutrients to sustain life. Scientists in favour of the “thrifty gene hypothesis”, suggest that humans are genetically predisposed to conserve energy, emphasizing the historic importance of ensuring energy intake and conservation when foods were available in order to survive in times of famine. From an evolutionary point of view, it was advantageous that numerous pathways were involved in ensuring adequate intakes, so humans could store large amounts of energy as AT for later use. The theory suggests the increasing prevalence of obesity has resulted as a lack of adaptation from a restrictive environment to one of plenty. Indeed, industrialisation and urbanisation have been proposed as important factors in the increasing prevalence of obesity sees in developing countries. Sometimes referred to as an obesogenic environment, an increase in the availability of energy-dense, lower-cost snacks and foods has contributed to the consumption of more calories, while more sedentary jobs, improved infrastructure, energy-saving devices (cars, trains, vacuum-cleaners etc.) and passive leisure activities (television, computers, cinema etc.) have dramatically reduced average daily energy expenditure of
individuals in industrialized areas. Cultural differences in body size ideals and preferences, and acceptance of adiposity, also exist between ethnic or social groups.

1.2. Obesity and cardiovascular disease

Obesity and overweight are risk factors for CVDs, type 2 diabetes mellitus, certain cancers and mortality. Overweight also exacerbates a number of other chronic diseases, including hypertension, dyslipidaemia, gallstones, osteoarthritis, obstructive sleep apnea and musculoskeletal problems.

Generally, health risks increase as BMI increases beyond 25 kg/m². Collaborative analyses of baseline BMI versus mortality in 57 prospective studies with 894,576 participants, adjusted for gender, age, smoking status and study, show that each 5 kg/m² increment in BMI above 25 kg/m², is associated with a 30% higher overall mortality risk; 40% increase in risk of vascular mortality, 60–120% increase in risk of diabetic, renal and hepatic mortality, 10% increase in risk of neoplastic mortality and 20% increase in risk of respiratory and all other mortality.

Recent evidence suggest obesity is associated with greater morbidity than smoking, alcohol and poverty, and may in near future exceed cigarette smoking as the leading preventable cause of death in the United States of America.

However, not all obese individuals present with increased CVD risk. A subgroup of obese patients present with normal metabolic profiles despite a high BMI or excessive adiposity. Although obesity is generally known to have adverse effects on blood lipoprotein concentrations, glucose tolerance and other metabolic pathways, not all patients present with metabolic disturbances. As many as 20% of obese individuals may present with relatively normal metabolic profiles, and without obesity-related risk factors, despite excessive adiposity. Little research has been conducted on the CVD risk in this subpopulation of obese individuals.

1.2.1. Adipose tissue – an active organ

AT is a specialized loose connective tissue, composed of adipocytes (fat cells), held in place by a framework of collagen fibers. It is the major storage site for fat in the form of triglycerides. AT is located mainly under the skin (subcutaneous AT), but also around internal organs (visceral AT). Two types of AT exist in humans, white AT and brown AT. White AT
predominates, and the tissue serves three functions: 1) energy storage, 2) heat insulation, 3) mechanical cushioning. As discussed in 1.1.3, fat provides a buffer for energy imbalances when intake does not equal energy expenditure\textsuperscript{36}, and human obesity is determined not by body weight, but by the amount of excess AT. Brown AT, on the contrary, releases energy directly as heat, and its main functions are heat production, non-shivering thermogenesis and diet-induced thermogenesis. Its main role is to maintain normal body temperature in infants, and the tissue regresses with increasing age, and is almost completely lost by adulthood. The following text will be referring to white AT.

AT consists primarily of large lipid-filled adipocytes, and approximately 60-85\% of AT, in weight, is lipid, of which 90-99\% triglycerides. Thus the size of AT mass is a function mainly of adipocyte number and size. However, the tissue also contains stromal-vascular cells, connective tissue matrix, pre-adipocytes, immune cells, including macrophages and leukocytes, and nerve tissue\textsuperscript{35,37}.

As lipids accumulate within adipocytes, AT mass increases by both hyperplastic and hypertrophic growth\textsuperscript{37}. The distribution of AT varies between individuals. Predominant accumulation of AT on the upper body is typically referred to as android, central or male distribution, while predominant accumulation in the lower body is referred to as gynoid, peripheral or female distribution. An individual’s fat distribution is determined mainly by genetic background, gender and age, while extreme weight gain and weight cycling may increase the accumulation of fat in the upper body region, including the visceral fat compartment. It is primarily the visceral fat compartment that is associated with risk of CVD, hypertension, insulin resistance (IR) and other diseases\textsuperscript{38}. Visceral fat is metabolically active, and as adipocytes enlarge and become activated with increasing weight gain, they recruit macrophages, and release various factors that promote inflammation and IR\textsuperscript{36}, creating a milieu that contributes to increased cardiovascular risk\textsuperscript{36}. Visceral fat has been associated IR, atherogenic dyslipidaemia (elevated circulating concentrations of triglycerides, smaller, denser low-density lipoprotein [LDL] particles and apolipoprotein B, and low concentrations of high-density lipoprotein cholesterol [HDL C]), hypertension and chronic subclinical inflammation\textsuperscript{39,40,41}.

AT has now been recognized also as an active endocrine organ. In addition to storing lipids, the adipocytes produce numerous bioactive peptides (adipokines\textsuperscript{38}). Adipokines are chemical compounds that play important roles in various physiological processes, and include
cytokines and cytokine-related proteins, complement and complement-related proteins, lipids and proteins involved in lipid metabolism and transport, enzymes involved in steroid metabolism, and other pro- and anti-inflammatory agents\textsuperscript{38, 42, 43}. These signalling molecules enable crosstalk between the cells within AT, and between AT and other organs, including muscle, liver and the brain. Thus adipokines can exert local, peripheral and central effects, by which AT modulates several bodily functions, including metabolism, body weight and inflammatory and immune status.

While some of these bioactive proteins contribute to the inflammatory and pro-atherogenic state associated with obesity, others have anti-inflammatory and protective properties. Leptin, an adipokine that has been extensively researched for its role in energy homeostasis, is thought to play a role in subclinical inflammation, IR and other pathological changes associated with increasing adiposity. Leptin will be discussed in 1.3.

\subsection{1.2.2. Anthropometry}

Both the total amount and the distribution of body fat have been discussed as factors that contribute the most to the risk of developing CVD\textsuperscript{44}. Body weight or BMI alone do not differentiate between lean- and fat mass, and do not accurately predict adiposity. Ideally, body fat % and distribution should be measured directly. Dual-emission X-ray absorptiometry (DEXA) is the current reference method for examining body composition. The principles underlying this method has been described elsewhere\textsuperscript{45}. The DEXA scan is costly, lasts approximately 12 minutes, and requires the subject is required to lie still in the supine position. As this is not plausible in large population studies, as well as weight restrictions on the equipment, various indirect measurements are used for best estimation of adiposity. Despite being a crude measure, BMI has proven predictable of CVD risk in large population studies\textsuperscript{6}. Studies have found that the risk of developing CVD increases linearly with obesity, classified by BMI and central adiposity\textsuperscript{23, 27, 46, 47}. The risk associated with increasing BMI was discussed in the previous section 1.2.

Central or visceral adiposity, measured by waist circumference or waist-to-hip ratio, have been emphasized as useful measures of obesity-related CVD risk. Studies have found that individuals that present with larger waist circumference, within the same category of BMI (under-, normal-, overweight, and obese class I, II, III), have higher risks of CVD compared to individuals with similar BMI but smaller waist circumference\textsuperscript{48}. The independent
prognostic values of BMI and central obesity on CVD events, in subjects with established CVD, was evaluated in a longitudinal study of a Canadian population (6,620 males, 2,182 females; mean age 66 years)\(^4^9\). The authors found that BMI was predictive of myocardial infarction only. When controlling for central adiposity, BMI had no predictive value. On the contrary, waist circumference (>98 cm in women and >103 cm in men) was an independent predictor of CVD death, myocardial infarction and total mortality.

Hip circumference, on the contrary, is indicative of peripheral fat deposition and appears to be inversely associated with CVD\(^4^4\). Because of the opposing risks of waist and hip circumferences, waist-to-hip ratio has become a common method for cardiovascular risk assessment. Findings from an 11-year prospective Australian mortality study, including 9,309 adults aged 20-69 years, suggest that waist-to-hip ratio was the clinical measure of obesity that best predicted all-cause- and CVD mortality\(^5^0\). The study reported that waist-to-hip ratio was far superior to BMI, as well as waist-to-height and waist circumference alone, in prediction of CVD mortality in both genders. A standardized case-control study, including 27,098 participants from 52 countries and representing several ethnic groups, reported that waist-to-hip ratio show a graded and highly statistically significant association with risk of myocardial infarction\(^4^9\). Another study, including 11,247 25-year old Australians, also reported that of various measures of obesity, the waist-to-hip ratio showed the strongest correlation with CVD risk\(^5^1\). However, after correcting for age, the correlations of BMI, waist circumference and waist-to-hip ratio with CVD risk were similar. Overall, there is no final consensus as to which anthropometric measurement is the most accurate in risk prediction, and it is likely that the best method depends on gender, age, ethnicity and other variables.

1.2.3. Dyslipidaemia

Obesity-related dyslipidaemia is characterised by elevated blood concentrations of triglycerides, reduced concentrations of HDL C, and an increased proportion of smaller, denser LDL particles\(^4^0\). Elevated triglyceride levels is an independent risk factor in the development of CVD\(^5^2-5^4\). Lipoproteins rich in triglycerides have a direct atherogenic effect, and increased levels also appear to be a marker of other potentially atherogenic changes, including smaller, denser LDL particle size, IR and elevated plasma concentrations of apolipoprotein B\(^5^3\).
Norwegian reference ranges for triglyceride concentrations are 0.45-2.60 mmol/L. Findings from the Second National Health and Nutrition Educational Survey II data, representing a random sample of adults from the United States within a wide range of socioeconomic status and age, show that obese men presented with an average increase of 1.1 mmol/L in triglyceride levels compared to normal-weight men, and obese women with an increase of 0.7 mmol/L compared to normal-weight women. A meta-analysis including 16 population-based prospective studies, reported that a 1 mmol/L increase in plasma triglyceride concentrations gave a 32% increase in risk of CVD in women, and a 14% increase in risk in men, independent of other risk factors.

The association between LDL C and obesity is less pronounced, however a change in the LDL composition occurs in obese individuals. An increase in small, dense, more atherogenic LDL particles, combined with a decrease in HDL C, has been reported to directly correlate with triglyceride concentrations. In response to elevated concentrations of triglycerides, these particles become enriched by very-low-density lipoprotein particles, which are triglyceride-rich lipoproteins synthesised in the liver, and then in turn lipolysed by hepatic lipase, resulting in smaller, denser LDL particles and a reduction in HDL2 C, the most variable and anti-atherogenic subspecies of total HDL C. Lipid-lowering medications, including statins, primarily lower plasma LDL C concentrations. These medications considerably reduce CVD risk. It appears, however, to be limits to the beneficial effect that can be obtained by reducing LDL C concentrations only, indicating that HDL may play an important role. HDL is believed to have anti-atherogenic properties, by participating in cholesterol efflux from macrophages in the arterial wall, and may also play a part in regulation of cell-adhesion molecules, as well as possessing anticoagulant-, anti-oxidative-, anti-inflammatory-, anti-aggregatory- and pro-fibrinolytic properties. Population studies have consistently reported that HDL C is a strong, independent inverse predictor of CVD. Reference ranges HDL C are 1.0-2.7 mmol/L. Data from Second National Health and Nutrition Educational Survey II, suggest that HDL C levels were reduced by 0.3 mmol/L in obese compared to normal-weight men, while a slightly larger difference was reported in women. In contrast, an analysis of four large population studies, concluded that an increase of only 0.03 mmol/L in HDL C concentrations is associated with a 2-3% decreased risk of future CHD, suggesting that depressed HDL C concentrations associated with obesity may confer significant risk.
Although weight loss improves lipoprotein abnormalities (triglycerides, HDL C) in obese patients, the distribution of AT appears to be an important factor in the association between obesity and dyslipidaemia. Central adiposity (i.e. a large waist circumference or a high waist/hip ratio) has been positively related to total cholesterol, triglycerides, HDL C (inversely) and increases in small, dense LDL particles after adjusting for total fat mass or BMI.

1.2.4. Inflammation

Obesity is associated with chronic subclinical inflammation. Subclinical inflammation is a state of low-grade inflammation, recognized by increased levels of inflammatory markers, that has not yet brought about recognizable clinical findings. Increasing adiposity is associated with elevations in circulating concentrations of inflammatory agents, including C-reactive protein (CRP). CRP is considered a non-specific biomarker of subclinical inflammation. The protein activates the complement system, thus plays a part in innate immunity. In healthy, normal weight populations, average circulating CRP concentrations are < 2 mg/L, while obese individuals typically present with higher concentrations. This primitive acute phase inflammatory protein is synthesized primarily by hepatocytes, and has a half-life of ~18 h, and is regulated by other cytokines, such as interleukin 1 and -6 (IL-6), tumour necrosis factor alpha and others. CRP is released in response to acute injury, infection or other inflammatory triggers, including inflammatory diseases, malignancy, necrosis and trauma, and can increase to 300 mg/L in conditions such as septic shock.

Inflammation is a risk factor of CVD. Inflammatory reactions are well known to induce and accelerate atherosclerosis, and subclinical inflammation has been suggested as the link between obesity and IR. As a marker of inflammation, elevated levels of CRP are consistently associated with increased CVD risk. CRP is predictive of cardiovascular events in apparently healthy middle-aged men and women, and with patients with a history of CVD. A recent study on CRP, obesity and incident of coronary heart failure in an overweight-obese population reported that coronary heart failure was not related to obesity in this population after adjustment for CRP, suggesting that CRP is an independent risk factor. The association between circulating CRP and CVD differs in diseases with (acute myocardial infarction) and without (angina, atherosclerosis) tissue damage. In the former, an acute phase reaction takes place due to tissue damage, thus CRP levels increase, while in the latter,
associations are based on baseline CRP concentrations. Circulating concentrations associated with increased CVD risk or worse course may therefore vary from cut-off points of 1.5 mg/dL in unstable angina to ≥ 20 mg/dL in acute myocardial infarction. It is debated whether CRP is simply an independent marker of a subclinical inflammatory process (such as atherosclerosis, or tissue damage in acute myocardial infarction), or if it directly contributes toward the development of CVD.

Another marker of subclinical inflammation is serum ferritin. Ferritin is an intracellular protein, and the principal iron storage protein. As an acute-phase reactant, ferritin is regulated by IL-6 and tumour necrosis factor alpha, the synthesis of which increases with inflammation and infection. Elevated ferritin concentrations have been implicated as a risk factor of CVD. Studies in normal-weight healthy individuals, have found associations between ferritin and adiposity, CRP, and lipids, and with IR and the metabolic syndrome.

It has been suggested that the primary activity of ferritin, in the inflammatory state, is to reduce iron bioavailability. Iron is a transition metal and can catalyze free radical formation. Enhanced formation of free radicals causes oxidative stress, which is implicated in the development of CVD. The theorized link between ferritin and CVD has been referred to as the “iron hypothesis”, which suggests that elevated concentrations of iron in tissues may increase the formation of highly reactive forms of oxygen free radicals which can lead to oxidation of LDL, and thus promote the development of atherosclerotic disease. Free radicals may also directly damage the endothelium, promote thrombosis and disrupt normal vasomotor function.

1.2.5. Insulin Resistance

IR, an attenuated biological response to insulin, is a common feature in obesity. Central fat accumulation appears to be the critical determinant of IR. Visceral fat, compared with peripheral fat cells, is more resistant to the metabolic actions of insulin and more sensitive to lipolytic hormones. A larger visceral fat depot is associated with an increased release of free fatty acids into the portal blood stream, which provides substrate for hepatic triglyceride synthesis, and possibly impairs insulin metabolism. The underlying mechanism of how excess visceral AT leads to whole-body desensitisation of insulin action is not completely understood, however IR and glucose tolerance are effectively improved with weight loss and physical activity.
IR is typically associated with obesity as part of metabolic syndrome\textsuperscript{100}. A common feature in obesity, metabolic syndrome is characterised by hypertension, glucose intolerance and dyslipidaemia\textsuperscript{101}. Each component of metabolic syndrome may contribute to the increased risk of atherothrombotic CVD observed in obese individuals\textsuperscript{102-104}. A recent study reported that 22\% of the variability in fasting glucose concentrations in a healthy population could be explained by BMI, and that the most glucose intolerant individuals presented with the most elevated insulin concentrations, i.e. were the most insulin resistant\textsuperscript{105}.

IR may cause hypertension in the obese, and has been associated with cardiovascular pathology including coronary artery disease, atherosclerotic plaque formation in the carotid wall, angina, elevated levels of fibrinogen and increased coagulability\textsuperscript{99}. Hyperinsulinaemia and glucose intolerance precede the development of diabetes type, 2 and obesity is a strong risk factor for type 2 diabetes\textsuperscript{106}. The development of a diabetes epidemic is becoming a major global challenge, closely following the obesity epidemic. Even at low levels of BMI, a linear correlation is observed between BMI and the incidence of type 2 diabetes mellitus\textsuperscript{107}, which in turn is a strong risk factor for CVD\textsuperscript{108}.

1.3. **Leptin**

Leptin is an adipokine that has received much attention in recent years. It is synthesized from AT, and circulating concentrations increase with adiposity\textsuperscript{36}. Leptin was first recognized for its role in regulation of long-term energy homeostasis, and more recently for being implicated in pathways involved in cardiovascular risk.

1.3.1. **Physiological importance**

Leptin is a 167-amino acid 16-kD signalling protein, also termed an OB protein. From the Greek \textit{leptos}, meaning thin, leptin has emerged as a key hormone in the regulation of body energy balance.

The hormone was discovered with the identification that a mutation in the leptin gene (\textit{ob}) was responsible for obesity in the \textit{ob/ob} mice\textsuperscript{109,110}. This was first demonstrated when it was reported that correction of leptin deficiency in the \textit{ob/ob} mouse, caused a marked reduction in food intake, and retraction of its obesity syndrome\textsuperscript{111-113}. Similar findings were later made in humans with congenital or relative leptin deficiency\textsuperscript{114-116}. Humans and rodents with a
mutation in the leptin gene or the leptin receptor gene (db/db), lack leptin signalling, and present with hyperphagia, reduced energy expenditure and a phenotype resembling a starvation state, including decreased growth and lean body mass, hypothyroidism, infertility and compromised immune function, in spite of obesity and type 2 diabetes mellitus with severe IR\textsuperscript{112, 113, 115, 116}.

Leptin is synthesised primarily in adipocytes in white AT, and circulating concentrations of the hormone increases proportionately with adiposity\textsuperscript{117}. Smaller amounts are produced in other tissues, including the hypothalamus, pituitary, stomach, mammary glands, placenta, testes, ovary, endometrium and other. The hormone has a functional and structural resemblance to pro-inflammatory cytokines, such as IL-6, and is classified as an adipokine or an “adipocytokine”. Leptin receptors are widely expressed in central and peripheral tissues, including the hypothalamus and the nervous system, vascular tissue, the myocardium, pancreas, monocytes and lymphocytes, and in skeletal muscle\textsuperscript{118}. Circulating leptin conveys information about the state of body energy repletion to the hypothalamus, which regulates energy intake and expenditure accordingly, to maintain energy homeostasis\textsuperscript{119, 120}. It appears that the main role of the hormone is to inform the central nervous system of reduced energy availability in energy-deprived states, and thereby stimulate energy intake and reduce expenditure to ensure sufficient energy is available for vital processes\textsuperscript{120-122}. Furthermore, adequate leptin levels are necessary to permit energy expenditure on tissue remodelling, growth and reproduction, and to regulate the autonomic nervous system, the immune system and elements of the endocrine system\textsuperscript{120-122}.

In recent years, leptin has been associated with several processes pertinent in CVD, including vascular function, dyslipidaemia, arterial pressure, inflammation, and IR. These associations will be discussed further in section 1.3.3.

\subsection{Mechanisms of action}

Leptin is secreted in a pulsatile manner, with marked diurnal-nocturnal variation\textsuperscript{123}. Serum leptin concentrations are highest between midnight and early morning and drop to the lowest point between noon and mid-afternoon. It has been suggested that the nocturnal peak may be due to cumulative hyperinsulinaemia during the course of a day, which is theorized to stimulate leptin secretion from AT\textsuperscript{124}. Although several factors may influence leptin secretion patterns, such as fat mass and fat distribution, gender and age, hormones and cytokines, short-
term energy intake\textsuperscript{125, 126, 127} and the size of the adipocyte energy stores\textsuperscript{128} appear to be the key agents in regulating serum leptin concentrations\textsuperscript{129}. Serum leptin concentrations in healthy, normal-weight individuals are typically in the range of 3-5 ng/mL, while concentrations in the range of 8-90 ng/mL are reported in obese individuals\textsuperscript{130, 131}.

Various neuronal and peripheral afferent signals interact to convey information to the hypothalamus about the status of energy balance\textsuperscript{132, 133}. Leptin signals information about the size of energy stores, i.e. adipose mass, from the periphery to the brain, where appropriate adjustments in efferent energy regulation pathways are initiated\textsuperscript{134, 132}. Increasing levels of leptin, signals that excess energy is being stored, which brings about adaptive processes that resist obesity, such as decreased appetite and increased energy expenditure\textsuperscript{124}. The availability of leptin to the central nervous system, is assumed to be regulated by the blood-brain-barrier, and a unidirectional satiable transport system has been confirmed in rats\textsuperscript{135} and mice\textsuperscript{136}.

Leptin-responsive neuronal populations have been distinguished in the arcuate nucleus in the hypothalamus\textsuperscript{137, 138}. It appears that pro-opiomelanocortin neurons, generating the anorexic peptide alpha melanocyte-stimulating hormone, which acts through melanocortin 3 and 4 receptors (MC4r), are the primary mediators of leptin action\textsuperscript{139}. MC4r-deficient rodents have severe obesity, show increased linear growth, and are hyperphagic and hyperinsulinaemic, while rodents that hare heterozygous for a null MC4r allele, present with intermediate weight gain compared with wild-type and homozygous mutant animals\textsuperscript{140}. A number of different mutations in the receptor have been reported in morbidly obese individuals, all of which cause a common non-syndromic form of obesity. Defects in this gene are among the most common genetic causes of obesity yet described, and it has been suggested that heterozygous coding mutations in the MC4r are implicated in 1-6% of cases of early onset to severe adult obesity\textsuperscript{141}.

In the hypothalamus, through a signalling cascade, leptin inhibits orexigenic neuropeptides, such as melanin-concentrating hormone, neuropeptide Y agouti-related peptide and orexins, while up-regulating anorexic neuropeptides, including alpha melanocyte-stimulating hormone, corticotropin-releasing hormone and cocaine- and amphetamine-related transcript\textsuperscript{133}. The overall effect is a reduction in appetite, increased sympathetic outflow and increased metabolic rate. The opposite is true when energy stores are low; leptin production declines, and thus the inhibiting action on production of neuropeptide Y and agouti-related peptide stops, while synthesis of alpha melanocyte-stimulating hormone and cocaine- and
amphetamine-related transcript falls. Neuropeptide Y release stimulates anabolic circuits, while agouti-related peptide antagonizes neuronal melanocortin receptors and thereby inhibits melanocortin signalling. This in turn leads to an increase in food intake and metabolic efficiency. Much of current knowledge of these complicated regulatory mechanisms has been obtained from rodent models of null mutations in the leptin gene (ob/ob mouse), the leptin receptor (db/db mouse), and genes encoding the MC4r and pro-opiomelanocortin neurons.

Leptin conveys its actions by interacting with its receptor (OB-R), which belongs to the class I cytokine receptor family, encoded by the diabetes (db) gene. Through alternative splicing of the single OB-R, multiple isoforms of the receptor are generated. In all species, isoforms can be categorised as short, long or secreted. Short isoforms are widely distributed in peripheral tissues, such as AT, skeletal muscles, liver and pancreatic β cells, supporting the pleiotropic roles of this adipokine, while both short and long isoforms are expressed in the hypothalamus. The short-form OB-R b is essential for leptin action. In fact, the leptin resistant db/db mice lack only the OB-R b, but closely resemble db/J/db/J mice in phenotype, which lack all forms of the OB-R, and the leptin deficient ob/ob mice. Leptin exerts many of its effects by acting on the central nervous system, and the OB-R b messenger ribonucleic acid is highly expressed in the hypothalamus. Here, leptin acts on neurons that are involved in the regulation energy homeostasis, as well as the regulation of circulating concentrations of a number of hormones, including thyroid hormone, growth hormone and sex steroids, and in the regulation of the autonomic nervous system.

Leptin circulates in free and bound form, and the soluble OB-R is the main leptin-binding protein in serum. The physiological function of free and bound leptin is not fully understood, and most studies examine total serum leptin rather than the subfractions. It has been reported that, in lean subjects, leptin circulates almost exclusively in the bound form (98%), while in obese subjects the opposite is true, and leptin is present mainly in the free form. It is hypothesised therefore, that the free form may be the more active form, as lean subjects do not require leptin to exert its inhibitory effect on food intake, while obese subjects present with elevated concentrations of free leptin. However, it has been reported that elevated leptin concentrations are indicative LR, and that leptin action is in fact inhibited in obese subjects, as will be discussed below.
More recent research suggests leptin also plays an important regulatory role in pathological processes involved in CVD risk. It has been suggested leptin has atherogenic effects of leptin on the vasculature, by interacting with receptors on the endothelium, vascular smooth muscle cells, macrophages and foam cells\textsuperscript{155}. Effects on the immune system appear to be exerted by direct interaction with haematocytes that contain OB-R b\textsuperscript{156}. Independently of its effects on adiposity, leptin appears also to be involved in the regulation of glucose homeostasis\textsuperscript{157, 158}, partly via effects on the central nervous system, and probably also by direct effects on pancreatic β-cells and insulin sensitive tissues. However, the exact mechanisms responsible for the association between leptin and various CVD risk factors have not yet been fully established.

**Leptin resistance**

While the administration of physiological levels of leptin, in case of congenital leptin-deficiency, will lead to sustained weight loss, in healthy normal-weight individuals with normal leptin concentrations, leptin administration has no effect on food intake or resting metabolic rate\textsuperscript{159}. The dose-response to increasing leptin concentrations peaks at physiological concentrations\textsuperscript{159}, and it appears that the major physiological role of the hormone may be to signal insufficient rather than excess energy stores, in order to ensure there is adequate energy available to sustain life.

Increased circulating leptin concentration is observed in obesity. The failure of elevated leptin levels to restore normal energy homeostasis is taken as evidence of LR in this population\textsuperscript{160, 161}. The exact physiology underlying LR is not fully understood. The causal mechanisms of LR may be a combination of impaired or saturated transport across the blood-brain-barrier\textsuperscript{162} and resistance at the level of the receptor and post-receptor signalling pathways\textsuperscript{163}. A change in the ratio of free versus bound leptin may also be implicated. Impaired leptin signalling results in hyperphagia and reduced energy expenditure in humans\textsuperscript{154}. This leads to not only to increased adiposity, and increased lipid storage in liver, muscles and other tissues, but also to alterations in the regulation of numerous neuroendocrine axes, including the thyroid, adrenal and reproductive axes, and the autonomic nervous system as well as the immune system\textsuperscript{154, 164}. It has been suggested that LR may be both a cause and a consequence of obesity, as increasing adiposity promotes LR, which in turn aggravates obesity, resulting in a never-ending cycle of increasing obesity\textsuperscript{165}.
LR is independently associated with IR and CVD\textsuperscript{118}. Obese individuals typically present with high circulating levels of both leptin and insulin, indicative of both LR and IR\textsuperscript{119, 160, 161, 166}. It appears that while obesity-related hyperleptinaemia, due to LR, fails to suppress feeding and mediate weight loss in obesity states, leptin sensitivity may be intact in peripheral tissues. Rather than absolute, LR may be anatomically distributed, and “selective”, in and that for example some centrally mediated effects of the hormone, such as the sympathetic responses, remain intact\textsuperscript{167}. Studies in genetic and acquired murine obesity models suggest that LR is restricted to the metabolic actions of leptin, while it’s cardiovascular sympathetic effects are still intact\textsuperscript{168}, and it has been speculated that selective LR may occur also in obese humans\textsuperscript{169}.

1.3.3. Leptin and cardiovascular disease

As discussed, the majority of obese patients have elevated leptin levels, and this has generally been associated with unfavourable CVD outcomes\textsuperscript{170}. Leptin appears to possess a number of actions that affect the cardiovascular system. A number of studies have implicated leptin in the pathophysiological processes leading to atherogenesis, atherosclerosis and obesity hypertension, and elevated serum leptin concentrations have been associated with a range of CVDs, including chronic heart failure, coronary heart disease, left cardiac hypertrophy, stroke and acute myocardial infarction\textsuperscript{167}. Leptin has consistently been associated with a number of established CVD risk factors, including adiposity, hypertension\textsuperscript{171}, dyslipidaemia\textsuperscript{172}, inflammation\textsuperscript{118} and glucose metabolism\textsuperscript{117}, as presented in figure 1.

There are strong correlations between leptin and measures of adiposity, including BMI, body fat % and waist-to-hip ratio\textsuperscript{173-175}. Independent associations between leptin and hypertension has been reported in normal-weight\textsuperscript{171} and obese subjects\textsuperscript{168, 176, 177}. Both pressor effects, including sympathetic activity increasing arterial pressure, and depressor effects, including increased endothelial nitric oxide, have been reported\textsuperscript{169}. Serum leptin has also been inversely correlated with plasma HDL C\textsuperscript{172} and positively correlated with triglycerides\textsuperscript{178}. Furthermore, the adipokine has been shown to increase cholesterol accumulation in foam cells and to increase oxidative modification of LDL\textsuperscript{167}. Studies have reported independent associations has been reported between leptin concentrations and intima-media thickness in the common carotid artery\textsuperscript{179}, decrease in arterial distensibility\textsuperscript{180}, and coronary heart disease\textsuperscript{181}, as well as the prediction of future cardiovascular events in subjects with established coronary atherosclerosis\textsuperscript{182}. Recent evidence has also suggested that leptin acts as a pro-inflammatory
cytokine, however the precise role of leptin in the development of inflammation is as yet not fully understood. A number of specific immune regulatory activities have been described, and leptin synthesis increases in response to sepsis, acute infection and a range of inflammatory mediators, including IL-6, CRP and fibrinogen. Conversely, chronic stimulation of pro-inflammatory cytokines appears to have the opposite effect, thus suppress leptin synthesis. Finally, leptin has been implicated in glucose metabolism, and is independently associated with insulin in normal-weight, overweight and obesity. Insulin resistant patients with diabetes type 2 present with elevated fasting leptin levels that correlate with fasting insulin independent of adiposity.

Figure 1. Associations between adiposity, leptin and CVD risk.
The figure presents the established relations between adiposity and CVD risk, and the associations in which leptin may be implicated.
Leptin in extreme obesity

Investigating the relations between leptin CVD risk in different populations, has resulted in paradoxical findings for the majority of associations\textsuperscript{170}. Most research has been carried out in normal- and overweight individuals. Less is known about the role of leptin in morbid- and extreme obesity and its association with the increased cardiovascular risk in these populations. The relation between adiposity and CVD increases in a non-linear manner, and although significant progress in the understanding of leptin as a contributing factor in the development of CVD has been made in recent years, the consequences of hyperleptinaemia and LR on cardiovascular physiology remain to be elucidated.

1.4. The MC4r Study

In April 2005 a study was initiated by the Department of Preventive Cardiology, Oslo University Hospital, Ullevål, to investigate the prevalence of mutations in the MC4r gene in a morbidly obese Norwegian population. The only inclusion criteria, was a BMI $\geq 30$ kg/m$^2$ at time of recruitment. Subjects were recruited during individual medical consultations at the Department of Preventive Cardiology, Oslo University Hospital, Ullevål, in Oslo. The study was based on data that was collected as part of the original medical consultations, including anthropometric measurements and blood samples.

The present master thesis is based on data from the MC4r study.

1.5. Rationale of the thesis

In light of the increasing prevalence of extreme obesity worldwide, and the health risk that may accompany this condition, we wished to investigate the relation of leptin with cardiovascular risk factors in a population of extremely obese adults. Further understanding about metabolic pathways and disturbances in extreme obesity, including CVD risk and contributing factors, is required, in order to increase knowledge about health risks associated with this condition, and to make preventive and remedial actions.
2. Aim, Objectives and Hypotheses

2.1. Aim

This thesis aims to assess serum leptin concentrations in an extremely obese population, and to investigate the cross-sectional relation between serum leptin concentrations and obesity-related cardiovascular risk factors in this population.

2.2. Objectives

The objectives of the project are to investigate the following:

- the association between fasting- and post-glucose challenge serum leptin concentrations and ethnicity, gender, body weight and measures of adiposity, ethnicity, smoking status, hypertension, type 2 diabetes mellitus and CVD.

- the association between fasting- and post-glucose challenge serum leptin concentrations and common CVD risk factors (smoking, blood pressure and HR).

- the association between fasting- and post-glucose challenge serum leptin concentrations and serum lipid concentrations.

- the association between fasting- and post-glucose challenge serum leptin concentrations and markers of inflammation (CRP and ferritin).

- the association between fasting- and post-glucose challenge serum leptin concentrations and markers of glucose tolerance and IR.
2.3. Null hypotheses

- There is no association between serum leptin concentrations and gender or ethnicity.

- There is no association between serum leptin concentrations and body weight or markers of adiposity (BMI, body fat %, waist circumference, waist-to-hip ratio or waist-to-height ratio).

- There is no association between serum leptin concentrations and common CVD risk factors (smoking status, blood pressure/hypertension and HR).

- There is no association between serum leptin concentrations and circulating concentrations of lipid related factors.

- There is no association between serum leptin concentrations and CRP and ferritin.

- There is no association between serum leptin concentrations and serum glucose, 2-h post-glucose challenge serum glucose, HbA1c, insulin, Homeostasis Model Assessment 2 indexes for Insulin Sensitivity (HOMA-S), Insulin Resistance (HOMA-IR) or steady-state beta-cell function (HOMA-β).
3. Methods

3.1. Subjects and Study Design

The present study relies on data collected in the MC4r study in Oslo, Norway, as described in section 1.4. The study design is outlined in figure 2.

This master-thesis studies a subgroup of the original MC4r cross-sectional study. The MC4r study was approved by the Norwegian Data Inspectorate and evaluated by the Regional Committee of Medical Research Ethics. The research was performed according to the declaration of Helsinki. Informed consent was obtained from all subjects.

The inclusion criteria in this master-thesis were 1) Age 18 to 65 years, 2) BMI $\geq 40$ at time of recruitment; and 3) fasting and postprandial blood samples available.
3.2. Data collection

Subjects were recruited between April 2005 and December 2010. At that time-point, 882 patients that had visited the clinic had met the inclusion criteria for the MC4r study (BMI ≥ 30 kg/m$^2$) and been included in the study database. All participants completed a health questionnaire of current health status and medical history. Blood tests were collected, and the subjects were screened for diabetes and glucose tolerance using an oral glucose tolerance test (3.5.1).

3.3. Anthropometry/Physical measurements

Trained healthcare professionals at the Division of Preventive Cardiology, Oslo University Hospital, Ullevål, carried out physical examinations. A number of anthropometric variables were measured.

Height and weight were measured with participants wearing only light clothing and without shoes. Height was measured to the nearest 1 mm using a stadiometer. Body weight was measured to the nearest 0.1 kg using a calibrated mobile electronic scale (Seca 720, Medical Scales and Measuring Systems, Birmingham, United Kingdom). Waist- and hip circumferences were measured with a constant tension tape measure. Waist circumference was measured at the midpoint between the inferior costal margin and the highest point of the iliac crest across the mid-axillary line, and hip circumference was measured at the widest point around the hips, both with arms relaxed on the sides. BMI was calculated according to the Quetelet formula as BMI = weight (kg) divided by the square of height (m$^2$) from weight measured in kg and height measured in cm.

Body fat % was measured with a leg-to-leg bioelectrical impedance analysis (BIA) scale (TANITA TBF 300 MA, Tillquist Lab. Med., Tanita Corporation, Tokyo, Japan). BIA is based on the principle that the electrical conductivity through body fluid is much greater in fat free compared to fat mass, as all body fluids and electrolytes are contained in fat free mass$^{184}$. Thus the scale determines the electrical impedance of an electrical current through the body tissues, which can be used to calculate an estimate of total body water. This estimate is used to predict fat-free body mass. Body fat % is calculated as the difference between body weight and total fat free mass. Two subdivided footpad electrodes are mounted on top of a platform scale, and the subjects stood barefoot on the scale. The subjects’ estimated clothes weight,
gender, age, height, body type (standard/athletic) and target body fat % were entered manually into the system. The leg-to-leg scale measures impedance across the lower limbs only. It is based on the concept that the whole body impedance is determined in large by the impedance of the arm and leg, thus the measurement of the impedance of a single extremity may be used to predict the impedance, and composition, of the whole body. According to the manufacturers, the Tanita Body Fat Monitor is accurate within ± 5% of the institutional standard of body composition analysis, the dual-energy X-ray absorptiometry. The precision is claimed at ± 1% variation when used under controlled conditions. The scale has a maximum weight limit of 200 g, and accuracy may be reduced for individuals who exceed 75% body fat.

Blood pressure and HR were measured automatically, using an automatic blood pressure monitor (52000 Series Vital Signs Monitor, Welch Allyn, New York, USA) or manually, using a mercury sphygmomanometer (CE0124, Big Ben Sphygmomanometer, round, Riester, Jungingen, Germany). Subjects were seated and rested for at least 5 minutes prior to assessment. The appropriate sized cuff, or tight cuff, was placed around the left upper arm. Three duplicate measurements were taken, and the average value of the two lowest measures was registered. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, and/or use of hypertensive medications.

3.4. Questionnaires

During the consultation, each patient completed two questionnaires, under the supervision of the health practitioner. One was a general medical information form, used in obesity consultations, including information about work status, personal history of cigarette smoking status, medical history, current medical conditions, including current use of medications, and family medical history (Appendix 1). Based on self-reported smoking status, the participants were classified into 3 groups: (i) current smokers, defined by smoking at least one cigarette daily, (ii) ex-smokers, (iii) those who never had smoked. The other questionnaire was a patient questionnaire, including information regarding civil status, personal weight history, including previous attempts of weight loss and use of medications or other weight loss aids, weight status of primary relatives, and alcohol intake (Appendix 2). Ethnicity was noted in patients’ journals as ethnic Norwegian and other non-Norwegian ethnicity.
3.5. Blood sample collections and analyses

Participants were instructed to fast overnight for a minimum of 10 h, during which they were instructed to drink only water and attended to provide a blood sample at a pre-specified time between 8:00 and 11:00. Five mL of venous blood was drawn according to standard protocol. Samples were centrifuged and refrigerated, and transferred to the Oslo University Hospital, Ullevål, Clinical Chemical laboratory, under cold chain conditions. Serum was stored at -80 °C, until leptin and insulin analyses were performed between November 2010 and May 2011.

3.5.1. Oral Glucose Tolerance Test

Baseline fasting serum glucose levels were measured after an overnight fast. After baseline fasting blood samples were drawn, patients ingested 50 g of carbohydrate drink. Blood samples were taken 2 hours after ingestion, from which 2-h post-challenge serum glucose was determined.

3.5.2. Biochemical analyses

All variables were analysed in serum using established methods. Analyses were performed at Oslo University Hospital Ullevål Clinical Chemical laboratory and Aker Endocrine laboratory.

Serum leptin was measured by an established human serum leptin Radioimmunoassay (RIA)\textsuperscript{185}. A Luminex instrument (Millipore, USA) was used for the analyses. The intra- and interassay variations of coefficients (CVs) were < 10%, and the recovery was 103-105% by the linear range of the assay. The detection limit of the assay was 0.5 ng/L (100 µL sample size). The assay had a specificity of 100% for human serum leptin.

Total cholesterol was determined by an enzymatic colorimetric method, using cholesterol esterase (Cobas Integra 800, Roche Diagnostics, Mannheim, Germany). HDL C was determined directly with a homogenous enzymatic colorimetric assay, using polyethylene glycol-modified enzymes in the presence of magnesium ions and dextran sulfate (Cobas Integra 800, Roche Diagnostics, Mannheim, Germany), and LDL C was calculated using Freidwals formula\textsuperscript{186}. Triglycerides were determined by an enzymatic colorimetric test, using lipoprotein lipase (Cobas Integra 800, Roche Diagnostics, Mannheim, Germany).
Apolipoprotein B was determined with an immunoturbidimetric assay on an automated analyser using sheep antibodies (Cobas Tina-quant 917, Roche/Hitachi, Roche Diagnostics, Mannheim, Germany).

Serum glucose was determined with the enzymatic reference method using hexokinase (Cobas Integra 800, Roche Diagnostics, Mannheim, Germany). Specific insulin was automatically determined by non-competitive immunofluorometric assay, using an AutoDelfia 1235 Automatic Immunoassay System (H1855-21291) (Parkin Elmer). HbA1c was measured automatically with HbA1c Calibrator Set using TSKgel Variant HSi with stable s-A1c (Tosoh Automated Glycohemoglobin Analyzer HLC-723G7, Tosoh Corporation, Japan).

IR was estimated according to the HOMA2. The HOMA2 calculator calculates indexes for insulin sensitivity (HOMA-S), beta cell function (HOMA-β) and IR (HOMA-IR) from fasting specific insulin (pmol/L) and fasting glucose (mmol/L). The model estimates steady state beta-cell function (HOMA-β), and insulin sensitivity (HOMA-S) as percentages of a normal reference population. The reciprocal of the index of insulin sensitivity, (100/HOMA-S), is calculated to give a score of IR (HOMA-IR). The HOMA2 calculator uses model-derived estimates, which take into account variations in hepatic and peripheral glucose resistance, the elevations in the insulin secretion at serum glucose concentrations exceeding 10 mmol/L, and the contribution of circulating proinsulin187. Higher HOMA-IR scores represent greater degrees of IR. The rationale undermining the HOMA2 score mathematical equation has been described previously188.

CRP was determined by particle-enhanced immunoturbidimetry with latex micro-particles coated with mouse monoclonal anti-CRP antibodies, (Cobas Integra 800, Roche Diagnostics, Mannheim, Germany). Serum ferritin was determined by an ADIVA Centaur analysis. The analysis is a double sandwich immunoanalysis using direct chemoilluminometric technology with a constant amount of two anti-ferritin antibodies, a goat polyclonal anti-ferritin and a mouse anti-ferritin bound with paramagnetic particles (ADIVA Centaur, Siemens Healthcare Diagnostics Inc., Tarrytown, USA).
3.6. Statistical analyses

Data was entered to the MC4r database in Epi Info, and prepared before analyses. Statistical analyses were performed using SPSS v.18.0 for Macintosh (SPSS Inc, Chicago, IL). Categorical data is presented as count and percentages, and continuous variables are expressed as mean ± standard deviation, SD. The study population was analysed in total, and in separate groups of females and males only.

All variables were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and logarithmically transformed values were used when indicated. Despite showing a skewed distribution, serum leptin change after glucose challenge (serum leptin Δ) could not be logarithmically transformed because the results included both positive and negative values. Non-parametric methods were used for analyses including this variable.

Chi Square tests were used to compare categorical variables between female and male subjects, and Students independent t-tests were applied to compare continuous variables. Gender differences in serum leptin were also investigated with a linear regression analysis after controlling for body fat. Pearson’s Correlations were performed to assess the independent correlations between log-transformed pre- and post-glucose challenge serum leptin concentrations and selected anthropometric measurements, lipids, inflammatory markers and indices of glucose tolerance. Males and females were compared. One-way analysis of variance (ANOVA) of independent samples was performed to compare individuals grouped into quartiles of selected parameters. Post-hoc tests using the Bonferroni correction were performed where the one-way ANOVA analyses reported statistically significant differences between groups. We also considered whether there was a gender difference by including gender and a gender x factor interaction term. General linear regression models with adjustment for gender and different body fatness parameters were used to evaluate the independent correlation of log-transformed serum leptin and selected variables. The partial correlation coefficient was used to describe the association between serum leptin and other continuous variables of interest, controlling for the effect of gender and body fatness. To further investigate the independent contribution of each selected variable, models were also developed which controlled for the contribution of other relevant variables.

\( P \)-values of < 0.05 (two-sided) were considered statistically significant.
3.7. **Student work**

This master thesis included the following tasks:

- entering data to the database
- database preparation
- blood sample handling and preparation, including alliquotation of samples
- utilization of various software programs (Microsoft word, excel, EndNote, SPSS)
- literature review
- statistical analyses
- data interpretation
- manuscript preparation
4. Results

4.1. Description of the study population

4.1.1. Demographics

Table 2 shows the demographic characteristics of study participants (62% females).

Table 2. Demographic characteristics of the study population

<table>
<thead>
<tr>
<th>Civil status</th>
<th>Females N=196¹ (%)</th>
<th>Males N=119¹ (%)</th>
<th>P-value</th>
<th>Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>76 (39)</td>
<td>39 (33)</td>
<td>0.487</td>
<td></td>
</tr>
<tr>
<td>Widow</td>
<td>2 (1)</td>
<td>1 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>36 (19)</td>
<td>17 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separated/divorced</td>
<td>18 (9)</td>
<td>11 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>63 (32)</td>
<td>51 (43)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Work status</th>
<th>Females N=196¹ (%)</th>
<th>Males N=119¹ (%)</th>
<th>P-value</th>
<th>Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-time</td>
<td>88 (46)</td>
<td>65 (55)</td>
<td>0.348</td>
<td></td>
</tr>
<tr>
<td>Part-time</td>
<td>16 (8)</td>
<td>5 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full sick leave/disability</td>
<td>36 (19)</td>
<td>20 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial sick leave/disability</td>
<td>11 (6)</td>
<td>5 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rehabilitation</td>
<td>23 (12)</td>
<td>17 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>18 (9)</td>
<td>6 (5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Females N=196¹ (%)</th>
<th>Males N=119¹ (%)</th>
<th>P-value</th>
<th>Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norwegian</td>
<td>168 (86)</td>
<td>115 (97)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Other²</td>
<td>28 (14)</td>
<td>5 (3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Missing: civil status: n=1 (f); work status: n=4 (f), n=1 (m).
²Other non-norwegian ethnic background

The mean age of our sample was 41 ± 11 years, and there was no significant difference between women and men (40 ± 11 vs. 42 ± 10 years).

At the time of data collection, 54% of all the study participants were either married or had a partner, while the remaining group were single, separated/divorced or widowers. Near half of the population were in a fulltime job, while 36% were on either full or partial sick leave or in rehabilitation. There was no significant difference between females and males with regards to civil status or work status.
Of the total population, 90.5% were of Norwegian ethnicity. The remaining participants were of other, not specified ethnic origins. There were significantly more individuals of non-Norwegian ethnicities among females than males, 14% vs. 3% respectively (p = 0.004).

### 4.1.2. Established CVD risk factors

Table 3 presents the prevalence of established CVD risk factors in the study population.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Females N=196 (%)</th>
<th>Males N=119 (%)</th>
<th>P-value Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>45 (24)</td>
<td>28 (23)</td>
<td>0.417</td>
</tr>
<tr>
<td>Former</td>
<td>41 (21)</td>
<td>33 (28)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>110 (56)</td>
<td>58 (49)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td>0.356</td>
</tr>
<tr>
<td>Yes</td>
<td>66 (34)</td>
<td>47 (40)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>130 (66)</td>
<td>72 (60)</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td></td>
<td></td>
<td>0.628</td>
</tr>
<tr>
<td>Yes</td>
<td>23 (12)</td>
<td>17 (14)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>173 (88)</td>
<td>102 (86)</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (4)</td>
<td>5 (4)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>189 (96)</td>
<td>114 (96)</td>
<td></td>
</tr>
</tbody>
</table>

1Missing: civil status: n=1 (f); work status: n=4 (f), n=1 (m).

Of the total study population, 23% smoked at the time of data collection, while another 24% were former smokers. There was no significant difference in smoking status when comparing females with males. The relation between smoking status and fasting serum leptin was also assessed in a multiple regression model controlling for body fat %, and there was still no significant relation between serum leptin and smoking status (data not shown).

36% of the total population presented with hypertension (SBP ≥160 mmHg and DBP ≥ 95 mmHg), 13% presented with type 2 diabetes mellitus, 4% presented with history of CVD. There was no significant difference in prevalence of these CVD risk factors between females and males.
4.1.3. **Subject characteristics**

The anthropometric characteristics of the study participants are presented in table 4.

Table 4. Anthropometric characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Females N=196¹</th>
<th>Males N=119¹</th>
<th>p-value t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S D</td>
<td>Mean</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167</td>
<td>7</td>
<td>180</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>127.0</td>
<td>15.8</td>
<td>147.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>45.8</td>
<td>4.9</td>
<td>45.6</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>126</td>
<td>13</td>
<td>140</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>131</td>
<td>11</td>
<td>128</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.96</td>
<td>0.13</td>
<td>1.10</td>
</tr>
<tr>
<td>Waist/height ratio</td>
<td>0.76</td>
<td>0.08</td>
<td>0.78</td>
</tr>
<tr>
<td>Body fat percentage</td>
<td>50.6</td>
<td>3.1</td>
<td>42.8</td>
</tr>
</tbody>
</table>

¹Missing: waist circumference: n=2 (f), n=1 (m); hip circumference: n=2 (f); waist-to-hip ratio: n=1 (m); waist-to-height ratio: n=1 (m); body fat %: n=18 (f), n=20 (m).

BMI (kg/m²) ranged from 40 to 64 kg/m² in the total population, with a mean BMI of 45.7 ± 4.6 kg/m². There was no significant difference in BMI between groups of females and males.

There were significant differences between females and males in measures of height, weight, waist circumference, hip circumference, waist-to-hip ratio, waist-to-height ratio, body fat %. Males were taller and heavier, and had a larger waist circumference, hip circumference, waist-to-hip ratio and waist-to-height ratio compared to females. Conversely, females had a significantly higher body fat % than males (p < 0.001).

Mean circulating concentrations of selected CVD risk markers and serum leptin are presented in table 5.
### Table 5. Cardiovascular risk factors and serum leptin concentrations in the study population

<table>
<thead>
<tr>
<th></th>
<th>Females N=196</th>
<th>Males N=119</th>
<th>p-value t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132</td>
<td>16</td>
<td>141</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>85</td>
<td>10</td>
<td>89</td>
</tr>
<tr>
<td>Pulse (rate/min)</td>
<td>75</td>
<td>10</td>
<td>78</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.3</td>
<td>1.0</td>
<td>5.2</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.32</td>
<td>0.91</td>
<td>3.15</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.28</td>
<td>0.30</td>
<td>1.06</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.64</td>
<td>0.78</td>
<td>2.20</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>1.0</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>11</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ferritin (mg/L)</td>
<td>74</td>
<td>65</td>
<td>234</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>6.2</td>
<td>2.2</td>
<td>6.3</td>
</tr>
<tr>
<td>2-hour postprandial glucose (mmol/L)</td>
<td>7.6</td>
<td>4.1</td>
<td>7.9</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>6.0</td>
<td>1.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>108</td>
<td>68</td>
<td>148</td>
</tr>
<tr>
<td>HOMA-S (%)</td>
<td>56</td>
<td>32</td>
<td>40</td>
</tr>
<tr>
<td>HOMA-IR (%)</td>
<td>127</td>
<td>60</td>
<td>152</td>
</tr>
<tr>
<td>Ferritin (mg/L)</td>
<td>2.6</td>
<td>2.9</td>
<td>3.3</td>
</tr>
<tr>
<td>2-hour postprandial leptin (ng/mL)</td>
<td>64.0</td>
<td>33.1</td>
<td>45.5</td>
</tr>
<tr>
<td>HOMA-IR (%)</td>
<td>58.2</td>
<td>28.2</td>
<td>40.1</td>
</tr>
<tr>
<td>Leptin change (ng/mL)</td>
<td>-5.7</td>
<td>15.0</td>
<td>-4.5</td>
</tr>
</tbody>
</table>

1 Missing: SBP: n=3 (f); DBP: n=3 (f); HR: n=6 (f), n=6 (m); total cholesterol: n=2 (f); LDL C: n=3 (f), n=8 (m); HDL C: n=1 (f); triglycerides: n=1 (f); apolipoprotein B: n=1 (f); CRP: n=1 (f); HbA1C: n=2 (f), n=1 (m); insulin: n=2 (f), n=4 (m); HOMA: n=5 (f), n=4 (m); fasting leptin: n=2 (f), n=2 (m); 2-h post-glucose challenge leptin: n=1 (f), n=3 (m), leptin change: n=3 (f), n=5 (m).

2 2-hour post-glucose challenge.
There were significant differences between females and males in measures SBP, DBP and HR. Males had higher blood pressures and HR in our sample. We also compared blood pressures in participants with and without previously diagnosed hypertension, according to the medical questionnaires. In females only, SBP and DBP were significantly higher in patients presenting with hypertension compared to normotensives (p < 0.001). Furthermore, we compared blood pressures and HR in hypertensive patients that used blood pressure lowering medications with patients that received no treatment. We found that male subjects taking medication had significantly lower DBP compared to hypertensives that received no treatment for hypertension. In the female group, females taking antihypertensive medication had significantly higher HR compared to individuals that received no treatment for hypertension (data not shown).

Study participants presented with mean serum lipid levels that were within the reference limits for the general, health Norwegian population. Total cholesterol concentrations ranged from 2.5 to 11.2 mmol/L, and the mean value in the total population was 5.3 ± 1.1 mmol/L. There was no significant difference between females and males. Mean circulating HDL C in the total population was 1.2 ± 0.31 mmol/L, and females had significantly higher HDL C concentrations than males. Mean circulating LDL C in the total population was 3.3 ± 1.0 mmol/L, and there was no significant difference in LDL C concentrations between females and males. Circulating triglyceride concentrations ranged from 0.5 to 7.7 mmol/L, with a mean value of 1.9 ± 1.0 mmol/L, and females had significantly higher levels than males. Mean concentrations of apolipoprotein B was 1.0 ± 0.2 g/L, and there was no difference between females and males.

Mean circulating concentrations of CRP in the total population was 10.7 ± 14.4 mg/L (range 1-174 mg/L), with no significant difference between genders. Our population presented with higher levels than the reference level for the general, healthy Norwegian population, which is < 5 mg/L. Circulating ferritin concentrations ranged from 4 to 832 mg/L in the total population, and males presented with significantly higher values than females (p < 0.001).

Mean fasting and post-glucose challenge serum glucose concentrations in the total population were 6.2 ± 2.1 (range 4.1-21.7) mmol/L and 7.7 ± 4.0 (range 1.9-27.7) mmol/L, respectively, and there was no significant difference between females and males. Mean HbA1c levels were 6.0 ± 1.1%, and did not differ between females and males. Circulating insulin concentrations
in the total population ranged from 22-566 pmol/L, and males had significantly higher mean insulin concentrations than females (p < 0.001).

The HOMA2 indexes were used to estimate insulin sensitivity, beta cell function and IR. In the total population, mean HOMA-S was 50 ± 30%, and females had significantly higher insulin sensitivity scores compared males (p < 0.001). In contrast, HOMA-β was significantly higher in males than females, 152 ± 62 vs. 127 ± 60% respectively (p = 0.001). Mean IR (HOMA-IR) scores in the total population was 2.8 ± 2.5, and males were significantly more insulin resistant than females (p = 0.021).

Fasting serum leptin concentrations ranged from 10.1 to 230.5 ng/mL and post-glucose challenge values ranged from 9.9 to 220.2 ng/mL. Mean values were 57.1 ± 33.1 and 51.4 ± 28.3 ng/mL, respectively. Both fasting and post-glucose challenge concentrations were significantly higher in females than males (p < 0.001). We also assessed the change in leptin concentrations between the fasted state and 2-h post-glucose challenge. The mean change in serum leptin concentrations in the total population was −5.3 ng/mL, and was similar for females and males.
4.2. Serum leptin concentrations in the study population

As shown in figure 3, the distribution of fasting and 2-h post-glucose challenge serum leptin concentrations in our population was highly skewed.

Figure 3. Distribution of leptin concentrations in the total study population
The figure on the left shows the distribution of fasting serum leptin concentrations, while the figure on the right shows the distribution of 2-h post-glucose challenge serum leptin concentrations in the total study population.

Kolmogorov-Smirnov and Shapiro-Wilk tests confirmed that fasting and post-glucose serum leptin concentrations were not normally distributed in this population. To obtain normal distributions, serum leptin values were logarithmically transformed (figure 4), and log-transformed values were used in all further analyses.
As shown in table 6, there was a significant difference between fasting and post-glucose challenge serum leptin concentrations, and fasting and post-glucose challenge serum leptin concentrations were strongly correlated (figure 5). Results were similar in the total population and when analyses were done for each gender separately (p < 0.001). In both genders, there was a significant reduction in serum leptin concentrations 2 hours after the 50 g glucose challenge (p < 0.001).

Table 6. Fasting and post-glucose challenge leptin concentrations in the study population

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Fasting leptin$^1$ (ng/mL) (SD)</th>
<th>Post-glucose leptin$^1$ (ng/mL) (SD)</th>
<th>Pearson’s correlation</th>
<th>p-value t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>57.0 (33.1)</td>
<td>51.4 (28.3)</td>
<td>0.934</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All</td>
<td>311</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>194</td>
<td>64.0 (33.1)</td>
<td>58.2 (28.2)</td>
<td>0.913</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Males</td>
<td>117</td>
<td>45.5 (29.6)</td>
<td>40.1 (24.8)</td>
<td>0.938</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^1$Fasting- and 2-h post-glucose challenge serum leptin concentrations have been logarithmically transformed. $^2$2-h post-glucose challenge.
Females had significantly higher fasting serum leptin concentrations compared to males \((p < 0.001)\). The zero-order correlation between gender and fasting serum leptin was \(R = 0.35\) \((p < 0.001)\). The general linear regression model of the total population show that body fat % explained most of the association. After adjusting for body fat %, there was still a weak but significant correlation between gender and fasting serum leptin \((R = 0.19, p = 0.001)\). Because of the strong correlation between fasting and 2-h post-glucose challenge serum leptin values, only fasting levels were included in further analyses.

Figure 5. Correlation between fasting and 2-h post-glucose challenge leptin concentrations
The figure shows the strong correlation between fasting and 2-h post-glucose challenge serum leptin concentrations in the total study population.
4.3. Associations between plasma leptin concentrations and CVD risk factors

4.3.1. Leptin and demographics and selected CVD risk factors

One-way ANOVA for independent samples was used to compare categories. Fasting or post-glucose challenge serum leptin concentrations did not differ between groups of study participants based on civil status or work status (data not shown). When comparing leptin concentrations in participants of non-Norwegian ethnicity with ethnic Norwegians, non-ethnic Norwegians had higher fasting serum leptin levels than did ethnic Norwegians, however the difference was not significant.

We compared fasting and post-glucose challenge serum leptin levels between groups according to self-reported smoking status. Findings are presented in table 7.

Table 7. Leptin concentrations by cigarette smoking status

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Fasting leptin(^1) (ng/mL) (SD)</th>
<th>P-value ANOVA</th>
<th>Post-glucose(^2) leptin(^1) (ng/mL) (SD)</th>
<th>P-value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.062</td>
<td>0.342</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>158</td>
<td>60.5 (41.6)</td>
<td></td>
<td>54.0 (35.0)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>71</td>
<td>49.2 (26.4)</td>
<td></td>
<td>47.2 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>73</td>
<td>57.5 (30.6)</td>
<td></td>
<td>51.3 (25.9)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Logarithmically transformed leptin concentrations were used in the one-way ANOVA.
\(^2\)2-h post-glucose challenge.

Mean serum leptin concentrations in groups divided by smoking status. Individuals that smoked at least one cigarette/d were categorized as current smokers.

One-way ANOVA for independent samples was used to compare categories. There was no significant difference in either fasting or post-glucose challenge serum leptin concentrations between current smokers, former smokers and participants that had never smoked in our population. Serum leptin concentrations in individuals that presented with hypertension, CVD and type 2 diabetes mellitus were compared with levels in individuals without the respective
conditions. Student’s t test for independent samples was used to compare categories. There was no significant difference in fasting serum leptin concentrations between individuals with diagnosed hypertension or CVD compared with individuals without the diagnoses (data not shown) in the total population, or when analyses were performed for each gender separately. Furthermore, there was no significant difference in fasting serum leptin concentration between subjects diagnosed with type 2 diabetes mellitus compared with non-diabetic subjects in the total population, but when analyses were performed for females and males separately, we found that males presenting with type 2 diabetes mellitus had significantly higher fasting serum leptin concentrations compared with non-diabetic males (30.2 ± 11.5 ng/mL vs. 47.9 ± 30.9 ng/mL, p = 0.012). In females, there was no significant difference between diabetics and non-diabetics.

As shown in table 8, there were no correlations between fasting serum leptin and blood pressure. HR was not correlated with fasting serum leptin when analysing the population in total, but there was a significant association in the male but not female group when analyses were done for each gender separately.

Table 8. Unadjusted correlations between blood pressure and HR and fasting leptin concentrations

<table>
<thead>
<tr>
<th></th>
<th>Fasting leptin&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All N=212&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>SBP</td>
<td>-0.10</td>
</tr>
<tr>
<td>DBP</td>
<td>0.07</td>
</tr>
<tr>
<td>HR</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<sup>1</sup>Fasting serum leptin values have been logarithmically transformed.
<sup>2</sup>Patients taking anti-hypertensive medications have been excluded (n=97).

In all analyses, results using post-glucose serum leptin concentrations were similar (data not shown).
4.3.2. Anthropometry

Correlations between selected anthropometric characteristics and CVD risk factors and fasting serum leptin concentrations were examined. Findings are presented in table 9.

Table 9. Unadjusted correlations between anthropometric characteristics and CVD risk factors and fasting leptin concentrations

<table>
<thead>
<tr>
<th></th>
<th>Fasting Leptin¹</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All N=311²</td>
<td>Females N=194²</td>
<td>Males N=117³</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>p</td>
<td>R</td>
</tr>
<tr>
<td>Age</td>
<td>-0.06</td>
<td>0.279</td>
<td>-0.07</td>
</tr>
<tr>
<td>BMI</td>
<td>0.25</td>
<td>&lt;0.001</td>
<td>0.26</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.32</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>-0.08</td>
<td>0.159</td>
<td>0.03</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>0.31</td>
<td>&lt;0.001</td>
<td>0.27</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>-0.26</td>
<td>&lt;0.001</td>
<td>-0.15</td>
</tr>
<tr>
<td>Waist-to-height ratio</td>
<td>0.06</td>
<td>0.292</td>
<td>0.06</td>
</tr>
</tbody>
</table>

¹ Fasting serum leptin has been logarithmically transformed.
²Missing: body fat %: n=17 (f), n=19 (m); waist circumference, hip circumference, waist-to-hip ratio, waist-to-height ratio: n=2 (f), n=1 (m); SBP, DBP: n=3 (f)

Body weight was not significantly correlated with serum leptin in the population as a whole, but when analyses were performed for each gender separately, there was a weak but significant correlation in both the female and male group.

The correlations between various measures of adiposity and serum leptin were examined. BMI and fasting serum leptin were significantly associated in the total study population and the significance of the correlation persisted when analyses were performed for females and males separately. There was also a significant correlation between body fat % and fasting serum leptin in the total population, however the association remained significant only in males when performing analyses for males and females separately. To further assess the association between BMI and leptin, we grouped participants in quartiles according to BMI (figure 6). Categories were compared using one-way ANOVA for independent samples. In the females but not males, there were significant differences in leptin concentrations between
individuals grouped according to BMI quartiles. The post-hoc test with Bonferroni adjustment confirmed that in females, there was a significant difference in between individuals in the lowest and highest quartile according to BMI (p = 0.001). The group with the higher BMI presented with higher fasting serum leptin concentrations. There was no significant gender interaction between BMI and fasting serum leptin (data not shown).

Waist circumference and fasting serum leptin were not significantly correlated in the total population, but when analysing females and males separately, the weak correlation between fasting serum leptin and waist circumference in males reached significance. There was a significant correlation between hip circumference and fasting serum leptin in the total population, and when analyses were performed for each gender separately, correlations were still significant, and equally strong, in both groups. Waist-to-hip ratio and fasting serum leptin were inversely and significantly correlated in the total population, but the significance of the correlation persisted only in females when analyses were done for each gender separately. In contrast, the correlation between waist-to-height ratio and fasting serum leptin was not significant in the total population, but when analysing females and males separately there was
a significant association between waist-to-height ratios and fasting serum leptin in the male group.

In all analyses, results using post-glucose challenge serum leptin concentrations were similar (data not shown).

### 4.3.3. Lipids

Table 10 presents the correlations between fasting serum lipid concentrations and fasting serum leptin levels in the total population, and in females and males separately.

<table>
<thead>
<tr>
<th></th>
<th>Fasting leptin&lt;sup&gt;1&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All N=281&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Females N=181&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Males N=100&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>p</td>
<td>R</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>–0.06</td>
<td>0.348</td>
<td>0.07</td>
</tr>
<tr>
<td>HDL C</td>
<td>0.26</td>
<td>&lt;0.001</td>
<td>0.14</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>–0.17</td>
<td>0.005</td>
<td>–0.14</td>
</tr>
<tr>
<td>LDL C</td>
<td>–0.09</td>
<td>0.164</td>
<td>–0.08</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>0.08</td>
<td>0.141</td>
<td>–0.13</td>
</tr>
</tbody>
</table>

<sup>1</sup>Leptin values have been logarithmically transformed.

<sup>2</sup>Patients taking cholesterol-reducing agents have been excluded (n=31). Missing: total cholesterol: n=2 (f); HDL C: n=1 (f); triglycerides: n=1 (f); LDL C: n=2 (f), n=7 (m); apolipoprotein B: n=1 (f).

Associations were assessed using Pearson’s correlations. There was no significant correlation between total cholesterol and fasting serum leptin in the total study population, or when analyses were performed in separate groups for females and males. In contrast, there was a significant positive correlation between HDL C and fasting serum leptin in the total population (R = 0.26, p < 0.001). The association with HDL C was stronger and only significant in males compared to females (R = 0.21, p = 0.024 vs. R = 0.14, p = 0.067). Fasting serum leptin concentrations in the population, divided by gender, when grouped into quartiles by HDL C concentrations are shown in figure 7.
Categories were compared with one-way ANOVA for independent samples. There was no significant difference in serum leptin concentrations between individuals grouped into HDL C quartiles in either females or males. Furthermore, there was no significant gender interaction between HDL C and fasting serum leptin.

There was a significant negative correlation between triglycerides and fasting serum leptin in the total population ($R = -0.17$, $p = 0.005$), but the association was no longer significant when analyses were performed for each gender separately. The association between LDL C and fasting serum leptin was not significant in the total population, or in either females or males when analysed separately. Similarly, no significant association was found between apolipoprotein B and fasting serum leptin in either the total population or in groups of females or males only.

In all analyses, results using post-glucose challenge serum leptin concentrations were similar (data not shown).
4.3.4. Inflammatory markers

Correlations between CRP and ferritin and fasting serum leptin are presented in table 11.

Table 11. Unadjusted correlations between inflammatory markers and fasting leptin concentrations

<table>
<thead>
<tr>
<th></th>
<th>All N=311</th>
<th>Females N=194</th>
<th>Males N=117</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>p</td>
<td>R</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.25</td>
<td>&lt;0.001</td>
<td>0.23</td>
</tr>
<tr>
<td>Ferritin</td>
<td>-0.31</td>
<td>&lt;0.001</td>
<td>-0.18</td>
</tr>
</tbody>
</table>

1Leptin, CRP and ferritin values have been logarithmically transformed.
2Missing: CRP: n=1 (f); ferritin: n=2 (f).

The correlations between CRP and ferritin and fasting serum leptin were assessed using Pearson’s correlations. There was a significant association between CRP and fasting serum leptin in the total study population, the association was stronger in females compared to males. To assess if leptin levels differed according to CRP levels in our population, we grouped the study population, males and females separately, according to quartiles of CRP concentrations (figure 8), and compared leptin concentrations between quartiles with one-way ANOVA. The one-way ANOVA for independent samples showed that in females but not males, there was a significant difference between mean serum leptin values in individuals grouped according to CRP quartiles. The post-hoc test with Bonferroni adjustment showed that females in the highest quartile of CRP concentrations had significantly higher fasting serum leptin concentrations than females in the lowest quartile of CRP values (p = 0.014). Gender interaction was tested, and found not to be significant.
There was a significant negative association between ferritin and fasting serum leptin in the total study population, and the correlation was still significant when analysing females and males separately. When comparing fasting serum leptin concentrations in the population, by gender, and according to quartiles (figure 9), the one-way ANOVA for independent samples showed that there were no significant differences in fasting serum leptin concentrations between individuals grouped by quartiles of ferritin concentrations in either females or males (data not shown). There was no significant gender interaction between serum leptin and ferritin concentrations.

In all analyses, results using post-glucose challenge serum leptin concentrations were similar (data not shown).

Figure 8. Fasting leptin concentrations by CRP quartiles
Figure showing fasting serum leptin concentrations grouped by CRP quartiles. The figure to the left represents the female population and the figure to the right represents the male population.
4.3.5. **Glucose tolerance**

Table 12 shows the association between selected indicators of glucose tolerance and IR and fasting serum leptin. Associations were tested with Pearson’s correlations. There was no significant correlation between either fasting or 2-h post-challenge glucose and fasting serum leptin in the study population in total, or in separate groups of females and males. The correlation between fasting serum insulin and fasting serum leptin were not significant in the total study population, but when testing males and females separately, a weak correlation between insulin and fasting serum leptin was detected in males, however the association did not reach significance. There was no significant correlation between serum leptin and HbA1C in the total study population, or in groups of females or males only.

We detected no significant association between percentage of steady-state β-cell function (HOMA-β) and fasting serum leptin. The correlation between IR (HOMA-IR) and fasting serum leptin was also not significant in the total study population, but when analysed as separate groups of females and males, a weak but significant correlation was detected in males but not females (R = 0.22, p = 0.03).
Table 12. Unadjusted correlations between glucose tolerance and insulin resistance and fasting leptin concentrations.

<table>
<thead>
<tr>
<th></th>
<th>All N=273&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Females N=172&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Males N=101&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R  p</td>
<td>R  p</td>
<td>R  P</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>-0.00 0.926</td>
<td>-0.10 0.205</td>
<td>0.16 0.118</td>
</tr>
<tr>
<td>OG GT</td>
<td>0.03 0.641</td>
<td>-0.03 0.744</td>
<td>0.13 0.209</td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.01 0.832</td>
<td>-0.01 0.928</td>
<td>0.18 0.070</td>
</tr>
<tr>
<td>HbA1C</td>
<td>-0.02 0.741</td>
<td>0.01 0.899</td>
<td>-0.01 0.907</td>
</tr>
<tr>
<td>HOMA-β&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.08 0.141</td>
<td>-0.13 0.062</td>
<td>-0.02 0.870</td>
</tr>
<tr>
<td>HOMA-IR&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-0.03 0.620</td>
<td>-0.03 0.661</td>
<td>0.22 0.030</td>
</tr>
</tbody>
</table>

<sup>1</sup>Leptin values have been logarithmically transformed.
<sup>2</sup>Patients taking insulin or antidiabetic agents have been excluded (n=45). Missing: insulin: n=3 (f), n=2 (m); HbA1C: n=2 (f), n=1 (m); HOMA: n=4 (f), n=2 (m).
<sup>3</sup>HOMA index of beta-cell function.
<sup>4</sup>HOMA index of IR.

Figure 10. Fasting leptin concentrations in quartiles of insulin resistance
The figures shows fasting mean serum leptin concentrations in groups by IR quartiles. The figure to the left represents the female population and the figure to the right represents the male population.
Figure 10 presents fasting serum leptin concentrations in groups, for males and females separately, according to degree of IR. One-way ANOVA for independent samples was used to assess the difference in fasting serum leptin concentrations in between quartiles of IR (HOMA-IR score). Quartile 1 refers to the least insulin resistant subjects, i.e. with the lowest scores of HOMA-IR. In the male but not the female group, there was a significant difference in fasting serum leptin values between individuals in different IR quartiles. The Bonferroni adjustment post-hoc test was performed in the male group. In males, individuals in the highest quartile of IR presented with significantly higher fasting serum leptin concentrations compared to individuals in the lowest quartile of IR ($p = 0.008$). There was no significant gender interaction in fasting serum leptin between quartiles determined by IR.

In all analyses, results using post-glucose challenge serum leptin concentrations were similar (data not shown).

### 4.4. Multiple regression of leptin and selected risk factors

The association between HDL C, CRP, ferritin, HOMA-IR and fasting serum leptin was tested in a multiple regression analysis, controlling for gender and various measures for adiposity. In table 13, we controlled for BMI, in table 14, we controlled for waist-to-hip ratio and in table 15, we controlled for body fat %. All tables show unadjusted zero-order correlations between leptin and selected variables, partial correlations after adjusting for gender and adiposity (model 1) and partial correlations after adjusting for gender, adiposity, and all other variables in the model (model 2).

Model 1 in table 13 shows that correlations between HDL C, CRP and ferritin and fasting serum leptin were strongly reduced but remained significant after controlling for gender and BMI. The association between CRP and fasting serum leptin was almost unaffected by the additional inclusion of the other variables in the model (table 13, model 2). Similarly, the correlations with HDL C and ferritin, however, were not affected by adding the other variables to the model. All correlations remained significant. There was, however, no significant association between HOMA-IR and fasting serum leptin in either model.
The correlations between HDL C, CRP and ferritin and fasting serum leptin were all significant after controlling for gender and waist-to-hip ratio (table 14, model 1). The correlation between CRP and leptin was only slightly reduced, and less affected by controlling for gender and waist-to-hip ratio compared to gender and BMI. Including all other variables in the model had only minor effects on the correlations (table 14, model 2). There association between HOMA-IR and fasting serum leptin became stronger and significant when including gender, adiposity and all other variables in the model (table 14, model 2).

Table 13. Association between selected variables and fasting leptin concentrations by linear regression analysis: BMI model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Zero-order R</td>
</tr>
<tr>
<td>CRP³</td>
<td>310</td>
<td>0.25</td>
</tr>
<tr>
<td>Ferritin²</td>
<td>309</td>
<td>−0.31</td>
</tr>
<tr>
<td>HDL C³</td>
<td>280</td>
<td>0.26</td>
</tr>
<tr>
<td>HOMA-IR²,⁴</td>
<td>268</td>
<td>−0.03</td>
</tr>
</tbody>
</table>

¹Model 1: adjusted for gender and BMI, Model 2: including all other variables in the table.
²Fasting leptin, CRP, ferritin and HOMA have been log transformed.
³Patients taking cholesterol reducing agents have been excluded (n=31).
⁴Patients taking insulin or anti-diabetic agents have been excluded (n=45).

The correlations between HDL C, CRP and ferritin and fasting serum leptin were all significant after controlling for gender and waist-to-hip ratio (table 14, model 1). The correlation between CRP and leptin was only slightly reduced, and less affected by controlling for gender and waist-to-hip ratio compared to gender and BMI. Including all other variables in the model had only minor effects on the correlations (table 14, model 2). There association between HOMA-IR and fasting serum leptin became stronger and significant when including gender, adiposity and all other variables in the model (table 14, model 2).

Table 14. Associations between selected variables and fasting leptin concentrations by linear regression analysis: waist-to-hip model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Zero-order R</td>
</tr>
<tr>
<td>CRP²</td>
<td>307</td>
<td>0.23</td>
</tr>
<tr>
<td>Ferritin²</td>
<td>306</td>
<td>−0.32</td>
</tr>
<tr>
<td>HDL C³</td>
<td>277</td>
<td>0.26</td>
</tr>
<tr>
<td>HOMA-IR²,⁴</td>
<td>267</td>
<td>−0.03</td>
</tr>
</tbody>
</table>

¹Model 1: adjusted for gender and waist-to-hip ratio, Model 2: including all other variables in the table.
²Fasting leptin, CRP, ferritin and HOMA have been log transformed.
³Patients taking cholesterol reducing agents have been excluded (n=31).
⁴Patients taking insulin or anti-diabetic agents have been excluded (n=45).
In table 15, similar analyses were performed, controlling for gender and body fat %. Results were similar to findings in table 13. The zero-order correlations between HDL C, CRP and ferritin and fasting serum leptin were all markedly reduced, almost by half, but remained significant after controlling for gender and body fat % (model 1), but not affected by including all other variable in the model. There was no significant association between HOMA-IR and fasting serum leptin.

In all analyses, results using post-glucose challenge serum leptin concentrations were similar (data not shown).
5. Discussion and conclusions

5.1. Main findings and comparison to previous literature

In agreement with previous studies, we observed that extremely obese individuals had markedly elevated serum leptin concentrations, and that females had higher levels compared to males. We also report a significant difference, but strong correlation, between fasting and post-glucose challenge serum leptin levels in our population. Based on this strong correlation, I have mainly focused on the fasting serum leptin data in this master thesis.

Among the CVD risk factors in this extremely obese population, we report that leptin was independently and positively associated with HDL C, and with IR in males. Furthermore, serum leptin was independently and positively associated with serum CRP, but independently and inversely associated with serum ferritin. Our findings, in this population of extremely obese individuals, suggest that the role of leptin in pathways involved in CVD risk in this population group requires further study.

5.1.1. Plasma leptin concentrations in extreme obesity

Both fasting and post-glucose challenge serum leptin concentrations were determined in this study of extremely obese individuals (BMI 45.7 ± 4.6 kg/m²). We report mean serum leptin values of 57.1 ± 33.1 and 51.4 ± 28.3 ng/mL respectively. Few studies have investigated leptin in extremely obese individuals (i.e. BMI ≥ 40 kg/m²). In agreement with the results of this study, others that investigated this population found that individuals with extreme obesity present with high circulating leptin concentrations\(^90\). Like us, Van Dielen and colleagues (2001) investigated a population with a BMI ≥ 40 kg/m² (n=28, 89% women), and noted mean serum leptin concentrations of 52.7 ± 19.8 ng/mL\(^{189}\). Lee and colleagues (2001) investigated a multiethnic group of 197 individuals with a mean BMI of 40 kg/m² (mean age 39.8 years), and found that mean fasting serum leptin concentration in this population was 59.0 ± 28.8 ng/mL\(^{90}\). Even higher serum leptin concentrations were reported in 32 Finnish subjects (69% females), with a mean BMI of 48.1 kg/m² and a mean age of 44.2 years. In this study, mean serum leptin concentrations were 79.0 ± 2.4 ng/mL\(^{190}\). The higher mean BMI of
the participants in the latter study, compared to the studies conducted by Van Dielen and colleagues and Lee and colleagues may explain why this Finnish population presented with higher serum leptin levels.

In agreement with previous studies, our results show that females have higher serum leptin concentrations than males (fasting serum leptin of $\sim 64$ ng/mL vs. $\sim 45$ ng/mL). The gender difference in leptin concentrations, is generally ascribed to women’s relatively greater fat mass compared to males. However, Marshall and colleagues (2000) suggest that methodological problems, including choice of body fat measure and statistical methods, are responsible for the difference between males and females. The authors recommend adjusting for body fat % and total lean body mass. Both adipose and lean masses influence total blood volume, which in turn affects the circulating leptin concentration. Because of gender differences in body composition, men generally being larger than women, women and men with the same body fat % will have dissimilar absolute amounts of total lean mass. Men therefore typically have a larger blood volume in spite of a similar body fat %, which may influence circulating leptin concentrations. The authors suggest that there is in fact little difference in leptin concentrations between genders at a given percentage of body fat, when taking both lean and adipose mass into consideration, and using log leptin values and regression analyses. In our study, log serum leptin values were used, correlations were analysed with regression, and we adjusted for body fat %. We only had data on lean mass on a sub-group of our population ($n=135$, of which 83% females) and with an overall lower BMI than the total group of subjects with BMI $\geq 40$ kg/m$^2$. We therefore chose not to include lean mass in our analyses.

In our study, there was no significant difference in fasting or post-glucose serum leptin concentrations between individuals of Norwegian or non-Norwegian ethnicity. Others have reported differences in leptin levels across ethnic groups, but large population studies have found that such differences are almost entirely related to variations between ethnic groups in terms of in adiposity and body composition. In our study, only 10% of the population were of non-Norwegian ethnicity, thus a significant effect was unlikely to be detected. There was however a significant gender difference, and almost all individuals of non-Norwegian ethnicity were females in our study. This difference in ethnicity between genders may have influenced our findings as it is established that there are variations between ethnic groups in terms of body composition, blood parameters and CVD risk.
5.1.2. Associations between fasting and post-glucose leptin concentrations

We found that serum leptin concentrations were significantly lower 2-h after ingestion of a 50 g glucose load, compared to fasting values, and that there was a strong and significant correlation between fasting and post-glucose challenge leptin concentrations.

Others have made similar findings in obese but not lean individuals. Imbeault and colleagues (2001) compared the postprandial leptin response over an 8-h period in lean and obese (BMI $33 \pm 4$ kg/m$^2$) men, after a high-fat meal. They found a significant increase in postprandial serum leptin concentrations in lean men, but reported that levels significantly decreased in the obese group$^{195}$. In contrast, Romon and colleagues (2003) found that serum leptin concentrations significantly increased in the 9-h time-period after a carbohydrate rich meal (300 g carbohydrate), but that the increase was lower in obese subjects ($n = 11$, BMI $\sim 50$ kg/m$^2$), compared to normal weight controls$^{196}$. The postprandial increase in serum leptin was not observed until between 1 and 2-h after meal ingestion in the obese group. The authors suggested that obesity may be associated with an impaired postprandial serum leptin response, or enhanced clearance, compared to normal weight individuals.

Because leptin has appetite-suppressing effects, we had expected to see an increase, rather than a decline, in serum leptin levels after glucose ingestion. We observed, however, an acute reduction in serum leptin after a 50 g glucose load, perhaps due to a diminished response to carbohydrate ingestion in extreme obesity, in line with findings by Romon and colleagues (2003). Another explanation could be that the carbohydrate load was not large enough to cause a significant response. It is also possible that a longer postprandial period would have been required to observe a rise in serum leptin levels in obese subjects, as observed by Romon and colleagues. Pratley and colleagues (1997) investigated the postprandial serum leptin response after a mixed meal in healthy Pima Indians, and found that 2-4 h after meal ingestion, serum leptin levels dropped by 8%, and then subsequently increased to baseline levels where they remained until 15 h into the fast, before they again began to progressively decline$^{197}$. As the meal was ingested in a fasted state in the morning, the authors suggested that the decrease in serum leptin concentrations after the meal may be attributed to a continuation in decline from the prior night’s acrophase, as part of leptin’s circadian rhythm. While some studies have reported declines after breakfast, midday or evening meals, others
have reported no difference in the postprandial serum leptin levels after morning or night time meals\textsuperscript{198}.

Pratley and colleagues (1997) also reported a significant relationship between serum leptin and insulin in the fasting and postprandial state, and suggest this may reflect a chronic effect of insulin on leptin production\textsuperscript{197}. If insulin affects leptin synthesis, the postprandial change in serum leptin will be influenced by the nutrient composition of the meal, which affects insulin production, as well as insulin sensitivity. A more marked postprandial leptin response would be expected in response to elevated circulating insulin concentrations. The effect of insulin on serum leptin concentrations in overweight men was examined by Saad and colleagues (1998), who found a dose-dependent increase in serum leptin within 30-60 min of insulin infusion\textsuperscript{199}. The insulin-induced increase in serum leptin was proportionately lower in obese, insulin-resistant men, supporting a role for insulin in the regulation of leptin. The relationship between leptin and insulin and IR is controversial, and will be discussed in 4.3.5.

### 5.1.3. Associations between plasma leptin concentrations and CVD risk factors

**Adiposity measures**

In our population, serum leptin was positively associated with several indices of adiposity, including BMI, body fat % and hip circumference and inversely with waist-to-hip ratio. In males but not females, serum leptin was positively associated with waist circumference and waist-to-height ratio.

Because leptin is synthesized in AT\textsuperscript{117}, there is a physiological relation between body fat and leptin. Thus the best measure of adiposity, in terms of detecting associations with leptin, is body fat %\textsuperscript{192}. However, in population studies, anthropometric measurements are typically used. Lee and colleagues (2001) found, like us, that in univariate analyses, BMI, hip circumference and body fat % were the strongest determinants of the variance in serum leptin concentration in extremely obese subjects, all independently predicting half of the variance, while waist circumference predicted one third of the variance, and waist-to-hip ratio only predicted 8\%\textsuperscript{200}. In multivariate analyses, they observed that a model including gender and BMI was most strongly associated with serum leptin values, and explained most of the variance in serum leptin ($r^2 = 0.63$), while age and other indices of body fat distribution made
almost no contribution to the remaining variability. Others have reported associations as strong as \( R = -0.8 \) between leptin and BMI, in extremely obese patients (BMI \( \sim 48 \text{ kg/m}^2 \))\(^{190}\).

Compared to these studies, we did not find such a strong correlation between leptin and BMI (\( R = 0.25, p < 0.001 \)). In our findings leptin was more strongly correlated with body fat % (\( R = 0.32, p < 0.001 \)) as a measure of total adiposity. However, when analysing this relation by gender, the female group showed only a weak and non-significant correlation between serum leptin and body fat % (\( R = 0.08 \)). In contrast, and comparable to Lee and colleagues (2001)\(^{200}\), we found stronger correlations between leptin and hip circumference (\( R = 0.32, p < 0.001 \)) than between leptin and BMI, and similar associations were seen when analyses were performed for each gender separately. We also found a negative correlation between leptin and waist-to-hip ratio (\( R = -0.26, p < 0.001 \)). Similar findings were made by Lonnqvist and colleagues (1997), who reported a strong negative relation between leptin and waist-to-hip ratio, independent of BMI\(^{201}\). The authors suggested that leptin may in fact play a protective role in the pathology of obesity, as it appears to be more strongly associated with peripheral fat distribution, which has little association with CVD risk.

These findings indicate that peripheral fat, rather than total fat, may be the strongest determinant for serum leptin levels in our population. In line with this, more leptin is synthesized from subcutaneous than from visceral fat\(^{202}\), and studies have found that serum leptin is more strongly influenced by the amount of subcutaneous- than intra-abdominal AT in lean and obese individuals\(^{202, 203}\). This perhaps explains the stronger associations with hip than waist circumference observed in both genders, and the lack of association with waist circumference in women. Increases in peripheral fat are more typical of female than male obesity. This may explain the gender difference in serum leptin despite controlling for overall adiposity. However, others have reported no association between serum leptin and visceral fat mass in healthy, normal-weight individuals\(^{204}\), and the relation requires further investigations. We did not find studies on this question in extremely obese populations.

**Smoking**

In this study, we found no significant difference in serum leptin concentrations between smokers (~24% of the population), former smokers and non-smokers. We observed a trend of current smokers having higher fasting serum leptin levels than former and non-smokers, but
the difference was not significant. There was still no significant association between serum leptin and smoking status after controlling for body fat %.

Because smokers tend to have a lower BMI but relatively more visceral fat than non-smokers\(^{205}\), one would hypothesize that smokers may have similar or lower serum leptin levels to non-smokers. Results from studies that have investigated associations between leptin and smoking status, have been inconclusive. Some studies in overweight and obese populations have reported that current smokers have lower serum leptin levels than previous smokers\(^{206}\), even after correcting for BMI\(^{207}\), while others, like us, reported no difference in serum leptin levels associated with smoking status. One study that reported lower serum leptin levels in smokers compared to non-smokers, after controlling for age and BMI, also observed an acute decline in circulating leptin in response to smoking one cigarette\(^{208}\). The authors suggested that this may be due to increased plasma catecholamine concentration in response to nicotine, which is known to reduce serum leptin levels.

### Blood pressure and HR

Serum leptin and blood pressure were not associated in the present study. Mean blood pressures in females and males in our study were 132/85 and 141/89 mmHg respectively. 36% were hypertensive, and 78% of hypertensive individuals used medication to reduce their blood pressure. Other studies have not mentioned the use of blood pressure reducing agents, however, most hypertensive individuals are treated for their condition, and it is likely that patients in other studies were also taking medication.

Others have reported an association between blood pressure, hypertension and leptin concentrations in normal- and overweight patients. Agata and colleagues (1997) found a significant correlation between blood pressure and serum leptin concentration, and reported that serum leptin concentrations were significantly higher in essential hypertensives, than in normotensive controls, after controlling for BMI, gender and age\(^{171}\). Hall and colleagues (2010) suggest that hyperleptinaemia, rather than dyslipidaemia or hyperinsulinaemia, may be a plausible explanation of the increased prevalence of hypertension in obese subjects\(^{176}\). Studies in rodents have shown that chronic administration of leptin raises arterial pressure over time\(^{130}\), however the mechanisms by which this occurs are not fully understood\(^{171}\). Several mechanisms have been proposed to explain the relation between leptin and blood pressure. It appears that sympathetic effects of leptin may be preserved in obesity, despite
resistance to leptin actions on body weight regulation, and leptin has been associated with hypertension in both lean and obese populations. It has been suggested that the association between hypertension and elevated serum leptin concentrations in obese subjects, may be related to the high prevalence of IR in this population, which in turn is implicated in hypertension, and that leptin therefore only indirectly plays a role in the development of hypertension. However, a study of 92 non-diabetic normal- and overweight subjects with essential hypertension, found a difference in serum leptin concentrations between hypertensive and normotensive males but not females, suggesting other pathways may be involved. In our study, there was no difference in serum leptin concentration between normotensive and hypertensive subjects in either gender.

We were unable to identify studies investigating the relation between serum leptin and blood pressure in an extremely obese population.

In males but not females, serum leptin was positively associated with HR. An increased HR is a CVD risk factor, and an independent predictor of cardiovascular and all-cause mortality, in healthy individuals as well as in patients with diagnosed CVD. In line with our findings, a study in healthy normal-weight subjects found that serum leptin was associated with HR, independent of adiposity and plasma insulin, in men (R = −0.3) but not women. The authors suggest the reason for this gender-specific effect may be that men are more sensitive to leptin actions, and to excitatory stimuli of the sympathetic nervous system, compared to women. However, a study in transplant patients challenges this theory. Winnicki and colleagues (2001) reported that serum leptin and HR were independently and positively correlated in heart transplant patients. This interaction could not be explained by the effect of catecholamines alone, as heart transplantation entails denervation of the cardiac sympathetic and parasympathetic nervous system. The authors therefore suggest a direct action of leptin on HR, possibly mediated through cardiac leptin receptors.

Lipids

Serum leptin and triglyceride levels were inversely associated in this population, while serum leptin was positively associated with HDL C. The association with HDL C remained after adjusting for adiposity, suggesting an independent relationship between leptin and the major anti-atherogenic lipoprotein.
Generally, obesity has been positively associated with triglycerides and inversely associated with HDL C. Few studies have investigated the independent associations between serum leptin and lipid levels. To the best of our knowledge, the relation between serum leptin and serum lipids in an isolated group of extremely obese individuals has not been investigated previously. This finding is of interest, as an estimated 20% of obese individuals have been classified as “metabolically healthy, but obese”, presenting with rather favourable cardiovascular risk profiles, including with lipid levels comparable to healthy, normal-weight individuals.

In earlier studies in normal- and overweight individuals, some authors have found no correlation between serum leptin and lipids, while others have reported significant inverse relationships between serum leptin and HDL C, and positive relationships between serum leptin and triglycerides. However, a number of studies have, like us, reported positive relationships between serum leptin and HDL C, as discussed below.

Liuzzi and colleagues (1999) studied clinical, anthropometric and metabolic determinants of serum leptin concentrations in 400 obese patients (71% females), with a wide range of BMIs (31-82 kg/m²). Comparable to our findings, their results show a positive association between serum leptin and HDL C, and an inverse association with triglycerides. The associations persisted after adjusting for both gender and adipose mass (kg) measured with BIA. The authors proposed that after accounting for absolute fat mass, lower serum leptin concentrations were associated with higher CVD risk. Popruck and colleagues (2005) investigated the association between serum leptin and serum lipid concentrations in 214 overweight (BMI ≥ 25 kg/m²) Thai adults (78% females). Positive correlations between HDL C and serum leptin were observed in overweight and obese subjects, but not in normal weight subjects. The authors did not suggest any explanations for their findings. In contrast, Lonnqvist and colleagues (1997) found no relation between serum leptin and dyslipidaemia in their study of extremely obese individuals.

HDL C is known to be cardioprotective, thus our findings are interesting in light of the increased cardiovascular risk associated with increasing obesity. Obese individuals are resistant to leptin action, however it appears, as discussed, that LR may be selective and affect only some pathways. It is therefore difficult to conclude whether leptin is in fact cardioprotective and has a beneficial effect on lipids in obese individuals, or, on the contrary,
if leptin has atherogenic effects on serum lipids, which increasingly obese individuals are protected against due to LR.

Inconsistent findings and paradoxical observations in different populations, suggest there may be other, yet to be unravelled, confounding factors, influencing the relation between serum leptin and HDL C.

**Inflammatory markers**

In this study, an independent positive correlation between serum leptin and serum CRP, and an independent negative association between serum leptin and serum ferritin, were identified. The strength of the association was markedly reduced, but remained significant after inclusion of gender and adiposity in the model. Our findings are suggestive of a modest association between leptin and subclinical inflammation in our population.

In agreement with our findings, others have reported a positive correlation between serum leptin and CRP. Van Dielen and colleagues (2001) reported a significant positive correlation between serum leptin and CRP (R = −0.5) in a small group of extremely obese subjects, mostly women. However, after correction for gender and BMI, the correlation was lost (R = 0.09)\(^{189}\). The sample size may perhaps be the explanation why they were not able to show a significant relationship between leptin and CRP, above the relation due to gender and body mass. Another possible explanation is that the result is mainly explained by the known association between adiposity and low-grade inflammation. In a relatively large study, including 553 subjects (66% females, mean age −43 years) classified as lean, overweight or obese, there was a significant correlation between serum leptin and CRP (R = 0.14), but in this study too, the correlation was lost when adjusting for BMI\(^{221}\). The authors concluded that there was no direct relation between leptin and CRP, and that the correlation merely reflected an association between CRP and fat mass.

In a study by Shamsuzzaman and colleagues (2004), including 100 healthy subjects (52% females) (BMI ∼22-27 kg/m\(^2\)), serum leptin and CRP were significantly correlated after adjustment for age, gender, BMI, waist-to-hip ratio, smoking and alcohol consumption\(^{222}\). Theses authors suggested however, that the association between CRP and leptin, both of which are elevated in obesity, may be explained by the increase in number and size of adipocytes. CRP is synthesized in the liver, primarily under the influence of pro-inflammatory
cytokines, including IL-6\textsuperscript{80}. IL-6 is produced in large by adipocytes\textsuperscript{223}, which is also the site of leptin production\textsuperscript{117}. The authors also suggested that a possible explanation may be that leptin indirectly increases CRP production, via its ability to induce IL-6 production, as has been shown in \textit{in vitro} studies\textsuperscript{224}, which in turn induces CRP production\textsuperscript{223}.

As shown in our and some other studies, the association between leptin and CRP appears to be independent of body weight and adipose mass, but the association is weak, and causality is uncertain. Interventional studies may help clarify the role of leptin in subclinical inflammation. Hukshorn and colleagues (2004) investigated the effect of chronic leptin administration during weight loss on inflammatory markers in a group of obese individuals (BMI \textasciitilde 29 kg/m\textsuperscript{2}). The study found that in the control group there was no change in CRP levels in response to weight loss, while in the group receiving chronic leptin administration during weight loss, CRP levels increased 2-fold, peaking at day 8, and remained elevated for the rest of the study period\textsuperscript{225}. Notably, the leptin administration merely compensated for the natural decline in serum leptin during weight loss. Except the effect on CRP, leptin appeared to have a slightly had a slightly beneficial (anti-inflammatory) effect on overall plasma levels of cellular and humoral inflammatory markers during the weight loss period. Further investigations are needed to clarify these findings.

If leptin induces CRP production, then extremely obese individuals could potentially be at high risk of CVD due to their strongly elevated serum leptin concentrations. Romero-Corral and colleagues (2006) examined the relationship between serum leptin, CRP and CVD risk, from data in 6251 participants from the Third National Health and Nutrition Examination Survey II\textsuperscript{226}. They found a significant correlation between serum leptin and CRP, between CRP and CVD, and between leptin and CVD. However, after adjusting for leptin, CRP was not associated with CVD. Yet, the risk of CVD increased the most in response to elevated concentrations of both serum leptin and CRP together.

We also observed a significant negative association between serum leptin and serum ferritin. As for CRP, the association persisted but was markedly weakened after correcting for measures of adiposity. To the best of our knowledge, no other study has examined the association between leptin and ferritin, and we could only find one other study that has investigated the relation between leptin and iron status. These authors hypothesized that leptin may play a role in iron metabolism in overweight subjects, as many of leptin’s biological
actions resemble IL-6, which is an important factor in the development of anaemia in chronic diseases due to its stimulatory effects on synthesis and release of the iron regulatory hormone hepcidin\textsuperscript{227}. This \textit{in vitro} study reported that leptin could directly regulate hepatic hepcidin expression, which could contribute to low iron status in overweight and obese subjects. Ferritin, however, was not discussed.

In our study, males and females presented with mean ferritin concentrations of $234 \pm 150$ mg/mL and $44 \pm 65$ mg/mL, respectively. Hence our subjects had normal levels compared to reference values (males: $25$-$300$ mg/L, females $10$-$200$ mg/L). Elevated circulating ferritin concentrations may be indicative of subclinical inflammation. In contrast to our finding, other studies have reported elevated levels of serum ferritin concentrations in obesity\textsuperscript{228,229}.

It has been suggested that elevated ferritin, in obesity, may be due to iron overload, which in turn, due to excess accumulation of iron in body tissues, promotes the generation of reactive oxygen species and may lead to tissue injury\textsuperscript{91}. Yanoff and colleagues (2007) reported a positive association between BMI and serum ferritin in their sample of 234 obese subjects. Furthermore, the obese population presented with low serum iron, transferrin saturation and mean corpuscular volume, indicative of risk of true iron deficiency, combined with elevated serum ferritin concentrations, compared with normal-weight controls\textsuperscript{228}. The aetiology of this relation is unknown, however it has been suggested that the inflammatory-mediated sequestration of iron in the reticuloendothelial system could, despite adequate or increased iron stores, contribute to the development of low iron status in this population\textsuperscript{228}. It is not known whether obesity is a risk factor for true iron deficiency, or if this particular finding in obesity is due to a functional iron deficiency or related to an inflammatory state\textsuperscript{228}. The authors concluded that the observed increase in ferritin in obese subjects is likely to be caused by chronic inflammation.

In contrast with findings by Yanoff and colleagues, we report an independent inverse association between serum leptin and ferritin in our extremely obese population.

Elevated ferritin levels have been reported as a potential CVD risk factor\textsuperscript{94,230}, although findings have been inconsistent\textsuperscript{96} and a study in 598 obese (BMI $\sim 32 \pm 5$ kg/m$^2$) Spanish subjects, with mean ferritin concentrations of $\sim 84 \pm 99$ ng/mL, reported that ferritin levels were higher in patients who satisfied the criterion for the metabolic syndrome, and were positively associated with waist circumference, triglycerides and IR\textsuperscript{231}. A study in Finnish
middle-aged men reported that men with serum ferritin ≥ 200 µg/L had a 2.2-fold risk factor-adjusted risk of acute myocardial infarction compared to men with lower ferritin levels. The association was stronger in men that also presented with elevated LDL C concentrations, suggesting the increased risk may be due to lipid oxidation. Others have not been able to confirm these results and have suggested that the observation in the Finnish study could be due to chance, or that Scandinavian populations may be unique in terms of their higher incident of coronary heart disease and relatively higher levels of ferritin, compared to other Western populations. For instance, a prospective study in a middle-aged French population reported a positive association between ferritin and BMI, total cholesterol, triglycerides and blood pressures, but no association with risk of ischaemic heart disease. Similarly, a large prospective cohort study, including 1604 participants, found no significant association between ferritin and any cardiovascular endpoints.

Further study is required to examine the relations between ferritin, leptin and CVD.

**Glucose tolerance and insulin resistance**

We found in our study that serum leptin was significantly correlated with HOMA-IR, an index of IR, but only in males. In the total population, the association was lost when controlling for gender and adiposity (BMI, waist-to-hip ratio, body fat %). Mean HOMA-IR scores in the total population was 2.8 ± 2.5, and males were significantly more insulin resistant than females. No official cut-off values exist for this variable, however normal insulin sensitivity would give a HOMA-IR score of 1.

Greco and colleagues (2002) assessed the relation between serum leptin and insulin sensitivity. In agreement with our findings, they reported a negative correlation between serum leptin and insulin sensitivity (HOMA-S) in their study of 20 extremely obese patients (R = −0.6). Others have, like us, reported a correlation between leptin and IR in normal weight and obese subjects, independent of BMI. Our extremely obese population presented with resistance to both insulin and leptin actions. Thus if a causal relation exists between the two parameters in the healthy state, the pathway may be distorted in this population, as discussed in section 5.1.2. The finding by Imbeault and colleagues (2001) and Romon and colleagues (2003) indicating that obese individuals do not show the same elevation in serum leptin after nutrient ingestion compared to normal weight subjects, supports this hypothesis. Cnop and colleagues (2002) suggest that the association between
elevated leptin and increasing IR may be due to a concurrent increase in both visceral and subcutaneous fat compartments, rather than causally linked. The authors reported an inverse relationship between visceral fat and insulin sensitivity (R = −0.7), while subcutaneous fat, in contrast to visceral fat, was strongly associated with leptin (R = 0.8). BMI does not differentiate between visceral and peripheral fat mass, perhaps explaining the inconsistent findings on the importance of adiposity in the relation between leptin and IR.

Despite extensive research, the mechanisms by which AT modulates insulin synthesis and sensitivity are as yet not fully established. Leptin and insulin appear to have overlapping functions as signals generated in proportion to adipose mass, that link body fat stores to adaptive modulations of feeding behaviour. Leptin and insulin receptors are both concentrated in the arcuate nucleus, where both increase hypothalamic pro-opiomelanocortin gene expression, while inhibiting the expression of neuropeptide Y. In the healthy state, they both act centrally to reduce food intake while deficiencies in either insulin or leptin signalling in the hypothalamus stimulate food intake.

A recent review suggests that leptin, in the healthy state, may activate pathways similar to insulin, thereby alleviating the increase in glucagon and growth hormone. This in turn may improve insulin sensitivity, reduce insulin production in pancreatic β-cells, and inhibit hepatic glucose production. Individuals with congenital leptin deficiency typically present with severe obesity, IR and glucose intolerance, and leptin administration can fully counter these metabolic defects. In vitro studies suggest that leptin in concentrations as low as 0.01 nmol/L suppresses insulin secretion from human pancreatic islands, while insulin increases leptin production. Obese individuals with severe IR typically present with high circulating concentrations of both leptin and insulin. Kiefer and Habener suggest an “adiposinular axis”, a bidirectional positive feedback loop, may exist between AT and pancreatic islets. If this is true, then in obesity, hyperinsulinaemia, preceding IR, may stimulate enhanced leptin production from an increased mass of adipocytes, and thus give rise to hyperleptinaemia, which in turn leads to LR. Due to LR, leptin then fails to down-regulate insulin, allowing insulin secretion to continue, aggravating the hyperinsulinaemia and IR in vicious circle. If leptin and insulin synthesis interact, the atherogenic state apparently linked with hyperleptinaemia in the obese could, hypothetically, simply reflect the atherogenic state causally associated with IR.
We observed a univariate relation between leptin and IR in males only, and the association was lost when adjusting for gender and adiposity. Like us, others have reported gender differences in the relation between leptin and IR. Panarotto and colleagues (2000) reported a gender-related difference in leptin concentrations between 48 overweight individuals with newly diagnosed type 2 diabetes, glucose intolerance and normal glucose metabolism, after controlling for BMI. They report, contrary to our results, that in women only, individuals with type 2 diabetes or glucose intolerance (IR) had 40% lower leptin concentrations compared to healthy women, and that there was an independent and positive correlation between leptin and fasting insulin, explaining 80% of the observed variability in fasting leptin, after controlling for gender, BMI and glucose. In our study there was no correlation between leptin and insulin or IR in women.

5.2. Methodological considerations

5.2.1. Study design

The study was a cross-sectional study, based on routinely collected data. The strengths of this study included a large sample of 315 extremely obese adults, of which both genders were well represented (61% women). The low participation burden ensured a high participation rate. To the best of our knowledge, no other study examining an exclusive population of extremely obese individuals has included a population of this size.

There are several limitations to this study. The main disadvantages is the cross-sectional design, i.e. the study provides no information about the causality of relations. Nor can it provide direct evidence as to whether these factors mediate the relationship between leptin and CVD. Moreover, cardiovascular disease is shaped by long-term exposure to risk factors.

However, the significant associations between leptin and several variables, which are independently associated with increased CVD risk (in particular HDL, CRP and IR), may contribute to the increased risk of CVD associated with leptin. Thus, longitudinal and interventional studies, examining the associations over time are required to obtain further knowledge about the causality of the relations that we observed in our study.
5.2.2. Selection bias

The population included in this study was not a random sample of the original MC4r study population, as we selected a sub-population of individuals aged 18-65 years, and which presented with a BMI $\geq 40$ kg/m$^2$ at time of recruitment. Thus, findings in our extremely obese population cannot be extrapolated to all individuals in the same age group.

Furthermore, the study population was not a strictly random sample of extremely obese adults, being influenced by response to referral for obesity treatment at the Department. Patients may be referred for treatment to Oslo University Hospital, Ullevål, from all areas of the country, however individuals from the Eastern areas of Norway are overrepresented. Furthermore, patients that were recruited to the study had agreed to get treatment for their obesity, and differences between patients that seek and do not seek help for their health issues have been reported earlier.\textsuperscript{245, 246} Moreover, all studies that rely on voluntary participation are subject to selection bias. Subjects that seek treatment for their obesity may have altered their diet or lifestyle. On the other hand, the population was referred to the clinic for obesity treatment, and individuals with concurrent health problems, including CVD and IR, may have been more likely to be referred, and to accept the referral and seek treatment for their health complaints. Thus the prevalence of CVD risk factors in this extremely obese population may in fact have been higher than in a random population of extremely obese individuals. Thus, whether our extremely obese population differ from a similar obese group in the general population is difficult to predict.

Moreover, we had no normal BMI control group with which we could compare our results. Considering that extremely obese individuals typically present with metabolic derangements and LR, comparing findings from our extremely obese population with observations in a healthy, normal-weight population would provide further information about the relation between leptin, LR and CVD risk factors.

5.2.3. Data collection and handling

All data was collected during the initial health consultation, by educated health professionals. Because of the long period of time during which participants were recruited, a number of health practitioners were involved in data collection. Despite following protocol for anthropometric measurements, inter-practitioner variation in anthropometric measurement
practices may be an issue\textsuperscript{247}. Moreover, data was collected only at one time-point, and findings, including body weight, waist circumference, blood pressure and haemostatic parameters, may have been influenced by day-to-day variation. Hydration status\textsuperscript{248}, hormonal state\textsuperscript{249}, stress\textsuperscript{250}, sleep\textsuperscript{251}, diet\textsuperscript{252}, caffeine\textsuperscript{253} and alcohol intake\textsuperscript{254} and other may have influenced measurements.

Because data, on which this master thesis relies, were already collected prior to the study initiation, the choice of parameters included in the database was predetermined. The timeline and extent of this thesis did not allow for further data collection or the repetition of tests where results were missing or errors were detected. Demographic variables that would have provided more detail to study population characteristics include level of education, physical activity, dietary habits and alcohol consumption. These factors are may influence leptin\textsuperscript{255} concentrations and CVD risk\textsuperscript{256} alike\textsuperscript{257}. With regard to smoking, underreporting by individuals is common\textsuperscript{258}. Moreover, “current smoker” was defined as 1 or more cigarettes daily, and quantity was not taken into consideration. Quantification would have improved the assessment of the relation between smoking status and leptin, and with CVD risk factor profile.

Data was registered in a database developed and programmed by researchers in the original MC4r study, which could not be changed or reorganized by the master student. Due to the large workload and the time-span of data collection, a number of people participated in the process of data registration, including the master student. No manual was provided for the data-entering process, thus the database was subject inter-individual differences in the understanding of the registration process (e.g. the use of decimals vs. rounding up numbers) as well as human error in terms of punching numbers. When preparing the database before statistical analyses, the student detected a large number of errors in the database. These were corrected to the best of our knowledge, or excluded from the study.

**Anthropometry**

At present, BMI is the standard tool for classification of obesity on population level\textsuperscript{16}. Despite a direct correlation between BMI, adipose mass, and health outcomes, the BMI classification is a crude measure and not a perfect tool as it does not measure fat mass directly\textsuperscript{259}, and an individual with a high BMI and a large proportion of lean tissue could be falsely classified as obese despite having a low percentage of body fat\textsuperscript{260}.
Waist circumference is a measure of central adiposity, and has been related to cardiovascular risk. Waist circumference better reflects visceral adiposity than does BMI, and has been reported to be a better predictor of adiposity in men compared with BMI. However, the importance of waist circumference diminishes with increasing BMI, and it has been suggested that at a BMI ≥ 35 kg/m², waist circumference does not add to the level of health risk determined by BMI only. Indexes were also calculated for waist-to-hip ratio and waist-to-height ratio, which are other measures of fat distribution that have proven to be good indicators of body fatness and CVD health risk.

Leg-to-leg BIA was used to assess body fat %. All our subjects were within the weight and body fat % limits of the scale. The precision and validity of the scale was discussed in section 3.2.1. Validation studies have found that the leg-to-leg BIA approach provides similar results as the conventional method using 4 gel electrodes, and leg-to-leg BIA has been found to correlate well with measurements using underwater weighing in normal weight and obese subjects. The method shows high reproducibility. A review of validity studies of BIA in overweight and obese subjects conclude that BIA is valid for individuals with BMI < 34 kg/m², but that the disproportion between body conductivity and body mass reduces the accuracy of BIA in obesity, and that most predictive equations are unable to predict static body composition. To the best of our knowledge, no validation studies have investigated an isolated group of extremely obese individuals. The advantage however of using BIA for the estimation of body composition, compared to the gold standard method for estimation of body composition, DEXA, is that the method is simple, non-invasive and less time consuming, and does not expose the subject to radiation. Moreover, the standard DEXA equipment has an upper weight limit of 136 kg. Notable, more than two thirds of our population exceeded the upper weight limit for DEXA.

**Blood samples and analyses**

Blood samples were analysed for standard parameters in a successive manner. After the initial analyses, blood was kept in a freezer of −80 degrees Celsius. For this master thesis, we had to reanalyze samples for fasting insulin and for fasting and post-glucose challenge serum leptin. Samples were thawed and aliquoted into smaller test tubes, and refrozen before transport to the endocrine laboratory for analyses. The repeated freezing and thawing of samples is unlikely to have affected the quality of the blood and analyses. The work was carried out
by the student and a colleague, both without experience in blood sample handling, thus increasing the risk of both random and systematic error.

In this thesis, total serum leptin, including free and bound fractions, were analysed. Others have discussed that there may be variations in the proportion of free vs. bound leptin according to adiposity, and that the free and bound form may have independent physiological roles\textsuperscript{153}. The various forms of the leptin receptor may also play a role in leptin action and resistance\textsuperscript{152}, and further study on the subfractions of leptin and the variants of its receptor alike may help clarify pathway-specific activities of the adipokine. This should be considered in future studies.

With regards to blood parameters, the inclusion of other inflammatory markers would have benefited our study. We only investigated CRP and ferritin. For instance, data on IL-6 concentrations, known to influence both leptin, CRP and glucose metabolism\textsuperscript{222}, would have been relevant in our study. Adiponectin, an adipokine associated with cardioprotective properties\textsuperscript{270}, would also have been a valuable measure.

\subsection*{5.2.4. Confounding factors}

In our multiple regression models, we adjusted for gender and adiposity. An independent relation between leptin and gender has consistently been reported, with females presenting with significantly higher leptin concentrations compared to males\textsuperscript{191}. A relation between leptin and measures of adiposity has been reported in lean as well as obese subjects\textsuperscript{36}. However, researchers do not agree on the best choice of adiposity measure. We therefore used different models in our study, in order to assess correlations after adjusting for three different measures of adiposity; BMI, waist-to-hip ratio and body fat %.

Despite controlling for two established confounding factors, a number of other confounding and covariate variables may have influenced our findings. Dietary intake, physical activity levels, including activity levels in the previous days, as well as hydration and hormonal state, may have influenced anthropometric measurements and blood parameters alike.
5.2.5. Statistical aspects

Univariate and multivariate analyses were used to assess the relation between selected parameters and serum leptin. Such analyses detect associations, but do not provide insight to causal mechanisms. Confounding factors outside our knowledge may be responsible for the detected association, thus we cannot claim to have found direct relationships between variables. To our advantage, we had a large database and were able to detect small but significant associations. The clinical relevance of some of these associations remains to be determined.

5.3. Conclusions

We report in our study of extremely obese individuals that serum leptin levels were substantially elevated compared to reference values, indicating that our population were severely leptin resistant. Measures of adiposity correlated with serum leptin levels, and correlations between serum leptin and peripheral fat distribution (hip circumference and waist-to-hip ratio), were stronger than correlations with general adiposity or visceral fat distribution. These findings contrasts with the greater cardiovascular risk associated with visceral fat. Women presented with higher levels than males, independent of adiposity, again underscoring that leptin does not strictly associate with increased cardiovascular risk.

One finding in this extremely obese population was that leptin was independently and positively associated with HDL C. The association between leptin and this cardioprotective lipoprotein, adds to the conclusion that elevated leptin concentrations may not always be associated with elevated CVD risk.

Another main finding was that leptin was positively associated with CRP, and negatively associated with ferritin, suggesting that LR in this population may be pathway specific or that leptin may possess pathway-specific pro- and anti-inflammatory properties.

Finally, with regards to glucose metabolism, we report that fasting serum leptin levels were significantly higher than post-glucose challenge serum leptin levels, and there was a strong correlation between the two samples. In contrast to previous reports, we observed a decline in serum leptin after glucose ingestion. Our extremely obese population demonstrated LR as well as IR. In men only, we noted an independent correlation between leptin and IR.
The clinical relevance of our findings is uncertain. Notably several of the observed associations were relatively weak, and sometimes present in only one gender. We cannot exclude that some of the associations are explained by residual confounding. Furthermore, given that LR occurs in obesity, it is difficult to directly link hyperleptinaemia with cardiovascular risk factors. We cannot eliminate the possibility that leptin may confer both cardioprotective and pathological activities in extremely obese individuals.

### 5.4. Future implications

- Findings should be confirmed in a random sample of extremely obese individuals, and compared with a normal BMI control group.

- The relation between leptin and regional fat distribution should be investigated further.

- The gender difference in leptin concentrations should be investigated further, taking into consideration both fat and lean masses, and total blood volume. Preferably, DEXA scan should be used for assessment body composition.

- Investigations of the causality of our findings should be conducted, and the independent association between long-term elevation in leptin concentrations and obesity-related outcomes, including diabetes and CVD end-points should be examined.

- New studies involving leptin in obesity should consider measurement of bound versus free leptin. The physiological role of the different fractions should be investigated.

- Studies on the distribution, and importance, of various leptin receptors should be conducted.

- Investigation of the clinical importance of elevated leptin concentrations in extremely obese individuals should be conducted.

- The clinical significance of elevated leptin in obesity and leptin resistance must be investigated.


100. Grundy SM. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. *Am J Cardiol.* 1999;83:25F-29F.


LEGKONSULTASJON FOR FEDME

På forhånd fyller pasienten ut pasient-spørreskjema, BES og TFQ

<table>
<thead>
<tr>
<th>Jobbsituasjon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulltidsjobb:</td>
</tr>
<tr>
<td>Sykmeldt:</td>
</tr>
<tr>
<td>Deltidsjobb:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Røykevaner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Røyker pasienten?</td>
</tr>
<tr>
<td>Nei: Ja: <em><strong>sigaretter daglig. Sluttet: År</strong></em>_</td>
</tr>
<tr>
<td>Bruk av andre tobakk-artikler: Pipe: Snus: Annet: Spesifiser:_______________</td>
</tr>
</tbody>
</table>
Sykdommer

Skjelettplager:  Hypertensjon:  HKS:  Podagra:  Søvnapne:

Dyspne:  Infertilitet:  DM I:  DM II:  Depresjon:

Gallestein:  Spiseforstyrrelser:  PCO:  Hypotyreose:  Annet:

Kommentar (eks Cushing, hypothalamustumor): .................................................................

.................................................................

Antall førstegradsslektninger med DM (type I+II): ............................................................

Høyde: ________  Vekt: ________  BMI: ________  Kroppsfett i %________


Andre funn (eks Acantosis nigrans): ____________________________________________

HUSK SLEKTSTRE!
Medikamenter
(Valgfritt for legen)

1. …………………………………………………………………………………………

2. …………………………………………………………………………………………

3. …………………………………………………………………………………………

4. …………………………………………………………………………………………

5. …………………………………………………………………………………………

6. …………………………………………………………………………………………

7. …………………………………………………………………………………………

8. …………………………………………………………………………………………

9. …………………………………………………………………………………………
## Appendice 2.

**PASIENT-KLISTREMERKE**

### Pasient-spørreskjema

**Navn:** ..................................................................................................................................................

**Yrke:** ..................................................................................................................................................

<table>
<thead>
<tr>
<th>Sivilstatus:</th>
<th>Enslig</th>
<th>Gift</th>
<th>Samboer</th>
<th>Enke/Enkemann</th>
<th>Skilt/Separert</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hvor gammel var du da du utviklet overvekt?</th>
<th>10 år eller yngre</th>
<th>Mellom 10 og 20 år</th>
<th>20 år eller eldre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Var det noe spesielt som utløste din overvekt?</th>
<th>Røykeslutt</th>
<th>År:……..</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Medisiner</th>
<th>Spesifiser: ..................................................</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Psykisk påkjenning</th>
<th>Spesifiser: ..................................................</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fysisk sykdom</th>
<th>Spesifiser: ..................................................</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Redusert aktivitet</th>
<th>Spesifiser: ..................................................</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Annet/Vet ikke</th>
<th>Spesifiser: ..................................................</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td></td>
</tr>
</tbody>
</table>
Tidligere konkrete forsøk på vektreduksjon:

- Ernæringsfysiolog
- Kurs (eks Roede) Spesifiser: ....................................................
- Fedmeoperasjon
- Pulverkurer Spesifiser: ....................................................
- Xenical
- Reductil
- Helsekostprodukter Spesifiser: ....................................................
- Postordreprodukter Spesifiser: ....................................................
- Annet Spesifiser: ....................................................

Hvor mange enheter alkohol drikker du pr. uke? ......................

En enhet alkohol = 1 glass øl (0.33cl), et glass vin (125 ml) eller et drammeglass (2 cl)

Hvordan bedømmer du din mors vekt etter skalaen nedenfor da hun veide som mest før 65 års alderen (ikke under graviditet). Velg det nærmeste alternativet:
Hvordan bedømmer du din fars vekt etter skalaen nedenfor da han veide som mest før 65 års alderen. Velg det nærmeste alternativet:

Dine søsken, helsøsken eller halvsøsken. *Har du ingen søsken gå direkte til neste punkt.*

Før opp alderen på dine sosken, og bedøm deres nåværende vekt ved å krysse av for den betegnelsen som passer best:

<table>
<thead>
<tr>
<th>Kjønn</th>
<th>Alder</th>
<th>Mager</th>
<th>Normal</th>
<th>Kraftig</th>
<th>Fet</th>
</tr>
</thead>
</table>

Dine barn (ikke adoptivbarn). *Har du ingen barn gå direkte til neste punkt.*

Før opp alderen på dine barn, og bedøm deres nåværende vekt ved å krysse av for den betegnelsen som passer best:

<table>
<thead>
<tr>
<th>Kjønn</th>
<th>Alder</th>
<th>Mager</th>
<th>Normal</th>
<th>Kraftig</th>
<th>Fet</th>
</tr>
</thead>
</table>
Hva er din høyeste vekt etter fylte 20 år? Skriv ned antall kg: ............... 

Eventuelle kommentarer: ......................................................................................................................................................
........................................................................................................................................................................................................
........................................................................................................................................................................................................
........................................................................................................................................................................................................