Examination of serum leptin- and lipid levels and gender differences in a population with symptomatic knee osteoarthritis

Fanny Elmberg

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Written by: Fanny Elmberg
Abstract

Background
Osteoarthritis (OA) is one of the most frequent causes of pain in adults, and the most common sort of arthritis worldwide. OA is common in non-weight bearing joints, and this fact suggests a systemic underlaying mechanism, rather than a mechanic phenomenon. High levels of leptin and lipids are two new found risk factors for OA, although the molecular mechanism underlying the association between lipids and OA is not defined. In previous studies results shows that levels of leptin and lipids tend to distinguish between gender, therefor it is important to look at each gender separately.

Aim
The aim of this study was to examine if there is a correlation between leptin- and lipid levels in symptomatic OA patients, and if there are any gender-differences in those levels.

Methods
In this study, data from an ongoing project at FoU Spenshult was used. Included in the study was 89 subjects (n\text{men}= 25, and n\text{women}= 64) aged 30 – 63 years, with symptomatic knee OA. Blood samples were collected at Hallands Sjukhus for determining lipid levels, radiographic examination of both knees was done by an experienced doctor at Bäckagård’s health care to confirm OA, and leptin was analyzed by enzyme-linked immunosorbent assay (ELISA) at FoU Spenshult.

Results
Data from this study states that there is a statistical significance between genders considering levels of triglycerides, HDL-C, LDL-C, and leptin. It was also observed that the analyze for the whole group only showed a weak correlation for leptin and triglycerides. While for women separately, correlations were observed for leptin and triglycerides, LDL-C and HDL-C. For men, leptin showed correlations with triglycerides and HDL-C.

Conclusion
Receiving more knowledge about systemic mechanisms underlaying OA and understanding differences between genders may lead to earlier disease detection. Further investigations on symptomatic knee OA will hopefully lead to improved preventive treatment to slow or reverse further progression. In turn, the burden for the society may be reduced, both physical, psychological and socioeconomic.
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Background

Epidemiology of osteoarthritis
Arthritis is a broad term covering over hundreds of joint related diseases (Massengale et al. 2012). The most common one worldwide is Osteoarthritis (OA), which is a chronic inflammatory disease with autoimmune pathogenesis. In 2002, 43 million adults suffered from arthritis, whereof 26.9 million aged 25 years and over suffered from OA (Amoaka and Pujalte, 2014). OA is one of the most frequent causes of pain, loss of function and disability in adults among the Western populations (Arden and Nevitt, 2006). Examinations has shown that radiographic OA occurs in the majority of people by 65 years of age and in nearly 80% of those aged over 75 years. In young individuals and athletes, the more time they spend engaging in recreational and occupational activities, the higher are the risk of injuries (Amoka and Pujalte, 2014). This, in turn leads to a higher likelihood of developing OA. The disease is from a general perspective, seen as a result from “wear and tear”. Young active individuals use their joints more and therefor, the risk is higher. The burden of OA is not only physical, but also psychological and socioeconomic (Litwic et al. 2013). Patient suffering OA are more susceptible to become psychological distressed compared to patients with other chronic diseases. Because of the high frequency in the population, the economic burden of OA is large.

Pathophysiology of osteoarthritis
OA is characterized by a painful degenerative joint disease in the cartilage and surrounding tissues, mainly caused by a disproportion between repair and breakdown of joint tissues (Issa and Griffin, 2012). Previous research of the pathogenesis of OA have focused initially on variations in articular cartilage (Man and Mologhainu, 2014). Articular cartilage consists of extracellular matrix and chondrocytes. OA occurs as a result when the metabolic activity of chondrocytes fails to maintain homeostasis between synthesis and degradation. Figure 1 presents articular structure changes during OA progression, where chondrocytes produce matrix degrading enzymes (Zhang et al. 2016). One of these enzymes is matrix metalloproteinase 13 (MMP13), which degrades A disintegrin and metalloproteinase with thrombospondin motifs 5 (Adamts-5) and collagen. This in turn targets the protein aggrecan, which role is to withstand compression in cartilage. The synthesis of these enzymes further worsens breakdown of articular cartilage. What causes imbalance in the homeostasis between synthesis and degradation is not known, though it has thought to be the primary change in OA
patients. Together with biomechanical stress and cellular changes, secondary changes occurs affecting the whole joint. More recent studies, looking at the whole joint including subchondral bone, menisci, ligaments, nerves and synovium, indicates OA as a multifactorial disease.

Figure 1. Knee articular structure changes during OA progression and cellular responses (Zhang et al. 2016).

OA can occur in any joint, although it is most common in knee, hip, hand and foot. There are both environmental risk factors and intrinsic risk factors causing OA (Zhang et al. 2015). Included in the environmental risk factors are to name a few; age, joint trauma, obesity, gender, mechanical stress, etcetera. OA progression and obesity are a widely studied association (Aspen, 2010). The fact that OA is common in non-weight bearing joints of obese persons, suggests a systemic underlaying mechanism, rather than a mechanic phenomenon. Studying biomarkers, such as proinflammatory mediators involved in different stages of OA, may help in understanding the process of the pathogenesis. Therefore, this study will examine intrinsic risk factors underlying OA.

Leptin

Previous studies by Mutabaruka et al. (2010) and Vuolteenaho et al. (2009) have proposed leptin as a biomarker for OA, since a correlation between proinflammatory mediators and OA was found. Leptin is a circulating hormone, produced by adipocytes, and encoded by the obesity gene. Its main function is to regulate food intake and energy expenditure by acting on hypothalamic cell populations (Zhang et al. 1994). It controls the volume of adipose tissues and the total body mass index (BMI). Leptin also plays a role in other physiological processes
such as lipid metabolism, immune function, bone formation and inflammation (Wang et al. 2012). The study by Wang et al. (2012) demonstrated that leptin alone, combined with pro-inflammatory cytokines, can directly induce cartilage degradation by inducing nitric oxide synthase. Further, leptin receptors have been found in articular cartilage, which may also associate the hormone in the pathogenesis (Teichtahl et al. 2005). A meta-analysis by Zhang et al. (2015) examined the clinical significance of leptin levels in OA patients. This study exhibited that increased leptin expression levels are related with disease severity in patient suffering from OA, especially among women.

**Lipids: Cholesterol, low-density lipoprotein, high-density lipoprotein and triglycerides**

An alternative, new found risk factor for OA may perhaps be high cholesterol levels (Farnaghi et al. 2017). The molecular mechanism underlying this association is not yet defined. Cholesterol is a type of steroid consisting of four linked hydrocarbon rings, which binds to one hydrocarbon tail and on the other end, one hydroxyl group (Walker, Hall and Hurst, 1990). It is synthesized in the liver and is also obtained from the diet. The main role of cholesterol in the body is to help build and maintain cell membrane, and to produce bile acids, which facilitate digestion and absorption of fats in the diet. Since cholesterol is a nonpolar substance, it needs to be transported in plasma by lipoprotein particles. Low-density lipoprotein cholesterol (LDL-C) are cholesterol-rich particles generally called the "bad" cholesterol. LDL-C delivers cholesterol to cells in the body. High-density lipoprotein cholesterol (HDL-C) called the “good” cholesterol, is involved in the reverse transport, carrying excessive cholesterol to the liver for disposal. Triglycerides are a type of fat, found in blood, consisting of glycerol and three fatty acids. It is also synthesized in the liver and obtained from the diet. The main role of triglycerides is to store unused calories and provide the human body with energy. Triglycerides, also being a nonpolar substance, needs to be transported by a lipoprotein called chylomicrons. Increased or decreased levels of cholesterol and triglycerides can occur as a result of abnormalities in the synthesis, degradation, and transport of their carriers (lipoproteins). Hyperlipoproteinemia is associated with an increased risk of atherosclerotic cardiovascular disease.

Previous studies by (Oliviero et al. 2012 and Ananth et al. 1993) have presented that the synovial fluid of patients with inflammatory joint diseases, such as OA, contains higher volumes of cholesterol compared with synovial fluid in normal patients. Several studies have
also reported that there is a link between hypercholesterolemia (high cholesterol in the blood) and OA (Martin et al. 1997, Stürmer et al. 1998 and Al-Arfaj, 2003).

**Correlation between leptin and lipids**

Leptin and lipid levels have shown a correlation in a study by Jaleel et al. (2006), were eighty women, forty normal controls and forty obese women were selected. Leptin levels were analyzed by enzyme-linked immunosorbent assay (ELISA). Triglycerides, LDL-C and HDL-C were determined by standard kit methods, Clinicon 4010. The result showed increased leptin levels, triglycerides, and LDL-C in obese women compared to the control group.

Another study by Tamer et al. (2002) examined the relationship between leptin and lipid levels in atherosclerosis. The result showed a positive correlation between total cholesterol (T-C) and LDL-C with leptin, whereas HDL-C and leptin showed an inverse relation. A study by Slama et al. (2015) examined the circulating levels of leptin and ghrelin in obese Tunisian women. They also investigated the correlations of these hormones with BMI, and lipids such as LDL-C and HDL-C. Result showed that leptin levels were positively correlated with LDL-C and BMI, and that leptin was negatively correlated with HDL-C.

**Gender differences**

That gender differences exists in the occurrence of knee OA are well documented (World Health Organisation, 2003). A higher prevalence of the disease among women in all ages has been reported all over the world, mostly based on radiographic surveys of populations. These examinations detect severe OA pathology and do not indicate symptoms or disability.

Gender differences in circulating leptin levels are also known. Studies by Kennedy et al. (1997) and Hellström et al. (2001), examined that for any given measure of obesity, leptin levels are two-three times higher in women than in men. The conclusion states that there are differences in both regulation and action of leptin between genders and that these alterations in leptin levels can lead to progression of several diseases. Hellström et al. (2001) provides evidence for two different mechanisms underlying gender differences in leptin levels. A higher production rate and a larger adipose tissue mass.

The lipid profile is similar for both genders until puberty, where lipid levels tend to diverge (Russo et al. 2015). Middle aged women have lower levels of LDL-C and total cholesterol than men. During menopause the LDL-C levels start to rise in women, more than in men. In older age groups women continuously shows higher LDL-C levels than men. In men, lipid
profile changes by ageing, while the lipid profile for women are affected by ageing and estrogen status.

**Measurement of leptin**
The majority of the studies mentioned above used ELISA to analyze leptin levels, which is also the method used in this study. The ones that did not use ELISA were Kennedy et al. (1997) and Slama et al. (2015), which instead used radioimmunoassay (RIA). ELISA was introduced in the 1970s (Leng et al. 2008). The typical double antibody sandwich ELISA enables accurate and sensitive detection of the antigen. Advantages with this method is its highly quantitative results and that the procedure is generally reproducible. Weaknesses with the ELISA method is that, it is largely dependent on the operator skills and experience, the antibody quality, and the kit manufacturer.

**Rationale**
Studies mentioned above have showed correlations between leptin and OA, lipid levels and OA, leptin and lipid levels between genders. There is lack of research looking at gender-differences on both leptin and lipid levels, especially on knee OA patients. Investigations looking at the correlation between those levels, genders, and their effect on OA are nearly non-existence. This study may be a step on the way of understanding how specific biomarkers are related to each other in patients with symptomatic knee OA. Additionally, to raise awareness what the profile looks like for these biomarkers between genders among patients. More knowledge about these systemic mechanisms and their gender differences may lead to earlier detection of knee OA and preventive treatment could be initiated to slow or reverse further progression.

**Aim and research questions**
The aim of this study was to examine if there is a correlation or how strong it is between leptin- and lipid levels, and if there are any gender differences in those levels. These are the two research question investigated:

- Are there any gender differences between expression of leptin and lipid levels in patients with symptomatic knee OA?
- Is there a correlation or how strong is it between leptin and lipid levels in patients with symptomatic knee OA, and is the correlation different between the two genders?
Subject information

Included in the study was 89 subjects (nmen= 25, and nwomen= 64) aged 30 – 63 years. The BMI range of the population reaches from 18 – 48 kilograms per m². Among women in this study, 47 % and among men 52 % has confirmed knee OA. Subjects were invited through advertisements in Hallands Nyheter, Hallandsposten, Sju Dagar, and Laholms tidning. The inclusion criteria were; aged between 30 – 63 years with symptomatic knee OA and with no signs of inflammatory rheumatic disease. Excluded in the study were subjects who experienced earlier knee accidents/surgeries (knee trauma).

Methods

Data collected in this study was retrieved from an ongoing project (“Detection and prediction of disease course in symptomatic knee osteoarthritis”) at FoU Spenshult – rheumatological research and development. The setup of this study was cross-sectional. Data was collected from the population at one occasion to investigate the variables of interest.

Blood samples was obtained at Hallands Sjukhus (the hospital of Halland), with their staff, systems, and procedures for determining following lipids; T-C, HDL-C, LDL-C, and triglycerides from plasma, analyzed by photometry (Cobas 8000, Roche).

Leptin levels was analyzed by sandwich ELISA, (Version 8.2 ALPCO, Salem, Massachusetts, USA). The manual for this assay procedure can be found in Appendix 1. The kit that was used in this study were for quantitative determination of leptin in human serum. The software used to analyses the samples through the microplate reader was Magellan™ - Data Analysis Software (Tecan Sunrise, Tecan trading AG, Männedorf, Switzerland). All samples in this assay, standards and controls included, were tested in duplicate to allow intra-assay variation.

Radiographic examination of the tibiofemoral joints and patellofemoral joint was implemented to diagnose patients with knee OA and divide the study population into two groups, radiographic and symptomatic OA. Patients in the radiographic OA group, was after the examination defined with knee OA. The other group, patients with symptomatic OA, was only having the characteristics of knee OA, but were not defined with the disease after the radiographic examination. This procedure was made at Bäckagård’s health center by an experienced doctor.
Statistical methods
The statistical software that were used for the following calculations were Statistical Package for the Social Sciences (SPSS) version 21. At first the Shapiro Wilks test were used to test the normality for cholesterol, triglycerides, HDL, LDL, leptin, BMI and age. Since the result showed that data was nonparametric, Mann-Whitney U test was used to test the null hypothesis for the variables between men and women. To analyze the linear correlation between leptin and the lipids for the whole group Spearman’s correlation test was used. By this test a correlation coefficient (r) was given, expressing the strength and direction of the linear correlation. From the r-value a determination coefficient (r²) was calculated, expressing how differences in one variable can be explained by a difference in a second variable. For Spearman’s correlation test the general guide that Evans (1996) suggests different r values, was used to determine the strength of the correlations.

- 0.00 - 0.19 “very weak”
- 0.20 - 0.39 “weak”
- 0.40 - 0.59 “moderate”
- 0.60 - 0.79 “strong”
- 0.80 - 1.0 “very strong”

Thereafter, the group was split to analyze if the correlation was different for each gender. By a Chi-square test the frequency of OA for both genders was calculated. For all tests, p < 0.05 was considered significant.

Ethical and social considerations
Ethical approval was obtained before start. The study followed the guidelines of the Helsinki Declaration. Written consent from the participants was obtained before start. (File reference number: EPN 2016-229 & 2017/253)

The result of this study may be able to help people with knee pain or symptomatic knee OA in the prevention of the development of knee OA. OA is as mentioned earlier the most common form of joint disease. The burden is not only physical, but also psychological and an extremely high economic cost for the society (Bitton, 2009). Being able to help people in an early stage of OA may save the society major costs. Furthermore, it will help people improve their quality of life, but also to be able to work for a longer period of life.
Results

Are there any gender differences between expression of leptin and lipid levels?

Table one below presents the result from Mann Whitney’s U test. The p-value confirm if there are a statistical significance between the median for both genders for each variable. Since a p-value < 0.05 was considered significant, triglycerides, HDL-C, LDL-C and leptin rejects the null hypothesis. For this data, it can be concluded that the concentration of triglycerides and LDL-C in the group of males, was statistically significantly higher than in the group of women. Conversely, the statistically significance was even higher for concentration of HDL-C and leptin in women than in men, where p < 0.001. This data states that there are significant differences in concentrations of leptin and the majority of the lipids measured in this study among men and women. Total cholesterol concentrations between genders showed no statistical significance.

Table 1. Result from Mann Whitney U test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Median (min – max)</th>
<th>Women Median (min – max)</th>
<th>Men Median (min – max)</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol [mm/L]</td>
<td>5.3 (3.5 – 8.7)</td>
<td>5.2 (3.5 – 8.7)</td>
<td>5.4 (4.0 – 7.6)</td>
<td>0.231</td>
</tr>
<tr>
<td>Triglycerides [mm/L]</td>
<td>0.8 (0.4 – 5.0)</td>
<td>0.8 (0.4 – 5.0)</td>
<td>1.0 (0.5 – 2.6)</td>
<td>0.032</td>
</tr>
<tr>
<td>HDL [mm/L]</td>
<td>1.7 (0.9 – 3.0)</td>
<td>2.0 (0.9 – 3.0)</td>
<td>1.3 (0.9 – 2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL [mm/L]</td>
<td>3.4 (1.8 – 6.2)</td>
<td>3.2 (1.8 – 6.2)</td>
<td>3.8 (2.8 – 5.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Leptin [ng/ml]</td>
<td>12.3 (0.8 – 97.7)</td>
<td>15.9 (0.8 – 97.7)</td>
<td>4.3 (1.0 – 25.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age [years]</td>
<td>54 (30 – 63)</td>
<td>54 (30 – 62)</td>
<td>52 (38 – 63)</td>
<td>0.373</td>
</tr>
<tr>
<td>BMI [m²/kg]</td>
<td>25.3 (18 - 48)</td>
<td>24.2 (18 – 48)</td>
<td>26 (23 – 41)</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Is there a correlation or how strong is it between leptin and lipid levels, and is the correlation different between genders?

Table two presents the result from Spearman’s correlation test. The different groups of lipids were measured against leptin to find out if there were any linear correlations. For the analyze of the total group (T), only a weak positive correlation could be seen for leptin and triglycerides (r = 0.24) assuming to Evans guide. After analyzing each gender separately,
further and stronger correlations were illustrated. For women following correlations could be seen, a moderate positive correlation for leptin and triglycerides \((r = 0.4)\), a weak positive correlation for leptin and LDL-C \((r = 0.23)\), and a weak negative correlation for leptin and HDL-C \((r = -0.25)\). For men, a moderate positive correlation could be seen for leptin and triglycerides \((r = 0.54)\), and a moderate negative correlation for leptin and HDL-C \((r = -0.41)\).

\(R^2\) expresses how differences in one variable can be explained by a difference in a second variable. This study presents generally low \(r^2\) values, with a highest variance of 30 percent for leptin and triglycerides in men. Low \(r^2\) values are not inherently bad, in this study were the variables of interest showed statistically significance, it is still possible to draw important conclusions how changes in one variable can be associated with changes in a second variable.

*Table 2.* Correlations between leptin and lipid levels in the total group and separated into sex. (M = Men, F = Female, and T = Total group)

<table>
<thead>
<tr>
<th>Leptin</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>T</td>
<td>M</td>
</tr>
<tr>
<td>(p)</td>
<td>0.005</td>
<td>0.002</td>
<td>0.03</td>
<td>0.54</td>
</tr>
<tr>
<td>(r)</td>
<td>0.54</td>
<td>0.4</td>
<td>0.24</td>
<td>0.13</td>
</tr>
<tr>
<td>(r^2)</td>
<td>0.3</td>
<td>0.14</td>
<td>0.06</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figures two to five, presents diagrams of Spearman’s correlation test for each gender separately. Instead of just analyzing the \(r\)- and the \(r^2\)- value, the proliferation of all the participants was shown. By looking at these findings, some interesting aspects appeared. In the diagram in figure two for leptin and triglycerides in women, two individuals have a strong effect on the inclination of the linear correlation. Another observation was, that in most of the diagrams there are some more and some less visible clusters of “dots”, that are collected in an upper and a lower area.
Figure 2. These two diagrams present the correlation between leptin and triglycerides for both genders. For men $r = 0.54$, $r^2 = 0.3$ and for women $r = 0.4$, $r^2 = 0.14$.

Figure 3. These two diagrams present the correlation between leptin and cholesterol for both genders. For men $r = 0.13$, $r^2 = 0.02$ and for women $r = 0.19$, $r^2 = 0.04$.

Figure 4. These two diagrams present the correlation between leptin and LDL for both genders. For men $r = 0.17$, $r^2 = 0.03$ and for women $r = 0.23$, $r^2 = 0.05$. 
These two diagrams present the correlation between leptin and HDL for both genders. For men $r = -0.41$, $r^2 = 0.17$ and for women $r = -0.25$, $r^2 = 0.06$.

**Discussion**

**Interpretation with previous studies**

The two articles mentioned in the background by Kennedy et al. (1997) and Hellström et al. (2001) comparing leptin levels between men and women, presented that for any given measure of obesity the concentration of leptin is 2-3 times higher in women than in men. The data from this study confirms the previous findings with an elevated leptin concentration in women compared to men, the level for women in this were 3.7 times higher than in men. Interestingly the median BMI for men were higher than the median BMI for women. All though, BMI does not provide a reliable value for body fat level. Another measurement for body composition such as dual-energy X-ray absorptiometry (DXA) or waist-hip ratio, would have been a more reliable method. A disadvantage of using BMI, is that the equation can overestimate or underestimate body fat levels. The formula uses height and weight, but it does not distinguish if the weight comes from fat or lean mass. Using for example DXA, would have been a more reliable method when comparing leptin level and body fat level.

The study by Russo et al. (2015) presented that middle-aged women had lower levels of LDL-C than men. But, during menopause LDL-C levels start to rise in women and in older groups women continuously shows higher levels compared to men. Comparing those previous findings to this study, a statistical significance was also seen. The population examined in this study where aged from 30 to 63 years, with a median age of 54 years for both men and women. Levels of LDL-C for men was significantly higher than in women. According to Russo et al. (2015) the lipid profile for women are affected by ageing and estrogen status.
During menopause, which women experience in an average age of 51 years, LDL-C levels rise. This study does not confirm this theory, since the median age of the group of women are 54 years and the LDL-C levels are lower than in the group of men with same median age. If this could have any relationship with OA needs further investigation.

Jaleel et al. (2006) showed in a study on 80 women, whereof 40 obese and 40 normal, a correlation between increased levels of leptin and triglycerides in the obese group compared to the normal group. In this study, made on symptomatic knee OA patients, the data confirms previous research. For both genders, leptin and triglycerides showed a positive correlation. Observing figure two above, and the diagram for women, there are two individuals having a strong effect on the inclination of the linear correlation. If these two individuals would not have participated in the study, the result would have been different, and a correlation would most likely not be seen for leptin and triglycerides in women. Further investigations needs be done to be able to draw statistically assured conclusions from this. Considering the $r^2$-value, the correlation between leptin and triglycerides presented the highest numbers for both men and women in this study (for men $r^2 = 0.3$ and for women $r^2 = 0.14$). These are generally low $r^2$-values, which demonstrate that for men 30 percent of the variation in leptin is explained by the variation in triglycerides. Previous studies have shown correlations between these two variables, for example Banks et al. (2004), presented that depressed triglycerides levels inhibit leptin transport at the blood-brain barrier. From this study with such small $r^2$-values it is not possible to draw conclusions, more evidence will be needed to confirm this theory.

In 2002, Tamer et al. examined patients suffering from atherosclerosis. They found positive correlations between leptin and T-C and leptin and LDL-C. Whereas a negative correlation was found for leptin and HDL-C. Recently in 2015, Slama et al. studied obese women and found matching correlations. Additionally, this study on a different population confirmed the previous findings, showing a negative correlation for leptin and HDL-C. Thus, as one of these variables increases the other one decreases. For leptin and LDL-C only a weak positive correlation was seen in the group of women and none in the group of men.

To validate result from this study further examinations on OA patients needs be done. The collected data shows that levels of leptin and lipids are different between genders, even in symptomatic knee OA patients. The only variable that was different in this study compared with previous research was LDL-C, which concentration was higher in men. The correlations between leptin and lipids agreed with previous investigations on obese populations and atherosclerosis patients. Besides for leptin and LDL-C, were only a weak correlation could be
seen for women. To make further analyses of why this correlation may be different in patients with symptomatic knee OA, more research will be needed.

**Method discussion**
The method used to obtain levels for T-C, LDL-C, HDL-C and triglycerides will not be discussed in this thesis. These measurements were done at Hallands Sjukhus according to well-standardized methods by experienced analysts. Also, the radiographic examinations determining OA, made by a doctor at Bäckagård’s health center will not be discussed. The ELISA method, which was used to analyze leptin is generally reproducible, though it is largely dependent on operator skills, experience and antibody quality (Leng et al. 2008). The analysts implementing this assay did not have a lot experience but were under surveillance of a supervisor throughout the entire laboratory session. Since this assay are dependent on the operator, there is a risk that another operator would have got different results. Although, the result in this study agrees with previous studies on leptin levels. The fact that all samples were tested in duplicates is a mainstay for obtaining quality and reliable data, and which also allows intra-assay variation. Samples showing variation in between duplicates was analyzed twice. The assay procedure started with method controls within acceptable limits recommended by the manufacturer, which also indicates that the assay has been implemented correct.

**Conclusion**
The result in this study presents that there are gender differences in levels of leptin and several lipids in symptomatic knee OA patients. It also shows that leptin correlates with following lipids; HDL-C, triglycerides and weakly with LDL-C. These findings are not revolutionary, similar result have been presented earlier but not on patients suffering from symptomatic knee OA. Receiving more knowledge about systemic mechanisms underlaying OA and understanding differences between genders may lead to earlier disease detection. Further investigations on symptomatic knee OA will hopefully lead to improved preventive treatment to slow or reverse further progression. In turn, the burden for the society may be reduced, both physical, psychological and socioeconomic.
References


Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J. M. (1994)

Leptin ELISA
For the quantitative determination of Leptin in human serum

For Research use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 11-LEPHU-E01
Size: 96 Wells
INTENDED USE
This kit is for the quantitative determination of leptin in human serum by an enzyme immunoassay method. This kit is for research use only.

PRINCIPLE OF THE TEST
The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for leptin is immobilized onto the microwell plate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin. During the first step, leptin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue colored product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stop solution, converting the blue color to a yellow color. The absorbance is measured on a microtiter plate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of leptin in samples and controls can be directly read.

RESEARCH APPLICATIONS
Human leptin is a 16 kDa, 146 amino acid residue, non-glycosylated polypeptide. Leptin is encoded by the OB gene. Its major source is adipose tissue, and its circulating concentrations indirectly reflect body fat stores.
Plasma or serum concentrations of leptin are increased in obese humans and strongly correlate with the degree of adiposity as expressed by percentage of body fat or body mass index. The recently discovered hormone leptin contributes to the regulation of energy balance by informing the brain of the amount of adipose tissue in the body. The brain may then make the appropriate adjustments in either energy intake or expenditure.
Many areas of leptin physiology remain to be investigated. The roles of leptin in metabolism, insulin sensitivity, as a potential therapeutic modality for weight loss as well as involvement in endocrine function are active areas of research. While the future for leptin as a therapeutic agent is not clear, its involvement in many areas of physiology undoubtedly makes this a new hormone which requires extensive study in the future to understand its physiology.

PROCEDURAL CAUTIONS AND WARNINGS
1. This kit is intended for research use only. It is not for use in diagnostic procedures.
2. Practice the following good laboratory practices when handling kit reagents:
   ① Do not pipette by mouth.
   ② Do not smoke, drink, or eat in areas where samples or kit reagents are being handled.
   ③ Wear protective clothing and disposable gloves when handling the samples and kit reagents.
   ④ Wash hands thoroughly after performing the test.
   ⑤ Avoid contact with eyes; use safety glasses. If contact occurs, flush with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Avoid microbial contamination of reagents.
5. A calibrator curve must be established for every run.
6. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
7. The controls (included in kit) should be included in every run and fall within established confidence limits.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
10. Improper procedural techniques, imprecise pipetting, incomplete washing, or improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
11. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
12. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
13. When dispensing the substrate and stop solution, do not use pipettes in which these liquids will come into contact with any metal parts.
14. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
15. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
16. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

LIMITATIONS
1. All the reagents within the kit are calibrated for the direct determination of leptin in human serum. The kit is not calibrated for the determination of leptin in saliva, plasma, or other samples of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric, or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only assay buffer may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. This kit is for research use only. It is not for use in diagnostic procedures.

SAFETY: CAUTIONS AND WARNINGS

POTENTIALLY BIOHAZARDOUS MATERIAL
All serum samples should be considered potential biohazards and handled with the appropriate precautions.

CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogen peroxide, and sulfuric acid. If contact occurs with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SERUM COLLECTION AND STORAGE
Approximately 0.1 mL of serum is required per duplicate determination. Collect 4-5 mL of blood into an appropriately labeled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human samples as potentially biohazardous materials and take appropriate precautions when handling.
REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
1. Precision pipette to deliver 20-100 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate washer (recommended)
6. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater
7. Centrifuge
8. Timer

REAGENTS PROVIDED
1. Anti-Leptin Monoclonal Antibody Coated Microwell Plate-Break Apart Wells
   Contents: One 96 well (12 x 8) monoclonal antibody-coated microwell plate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2-8°C
   Stability: Unopened at 2-8°C until expiration date on label.

2. Monoclonal Anti-Leptin-Biotin Conjugate
   Contents: One bottle containing a monoclonal anti-leptin antibody conjugated to biotin in a protein-based buffer with a non-mercury preservative.
   Volume: 10 mL/bottle
   Storage: Refrigerate at 2-8°C
   Stability: Unopened at 2-8°C until expiration date on label.

3. Streptavidin-HRP Conjugate Concentrate
   Contents: One vial containing streptavidin conjugated to horseradish peroxidase in a protein-based buffer with a non-mercury preservative.
   Volume: 0.4 mL/vial
   Storage: Refrigerate at 2-8°C
   Stability: Unopened at 2-8°C until expiration date on label.
   Preparation: Dilute 1:50 in assay buffer before use (e.g., 40 µL of concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of concentrate in 12 mL of assay buffer. Discard any remaining solution.

4. Leptin Calibrators
   Contents: Seven vials containing leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of leptin. Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration</th>
<th>Volume/Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator A</td>
<td>0 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator B</td>
<td>1 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator C</td>
<td>5 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator D</td>
<td>10 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator E</td>
<td>20 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator F</td>
<td>50 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator G</td>
<td>100 ng/mL</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.
5. Controls
Contents: Two vials containing leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of leptin. Refer to bottle label for expected value and acceptable range. Volume: 0.5 mL/vial
Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.

6. Wash Buffer Concentrate
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used, dilute 50 mL of the wash buffer concentrate in 450 mL of water.

7. Assay Buffer
Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Volume: 20 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.

8. TMB Substrate
Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.

9. Stop Solution
Contents: One bottle containing 1 M sulfuric acid.
Volume: 6 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.

ASSAY PROCEDURE
All reagents must reach room temperature before use. Calibrators, controls, and samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the streptavidin-HRP conjugate and wash buffer.
2. Pipette 20 µL of each calibrator, control, and serum sample into the correspondingly labeled wells in duplicate.
3. Pipette 80 µL of the monoclonal anti-leptin-biotin conjugate into each well.
4. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
5. Wash the wells 3 times with diluted wash buffer (300 µL/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of an automatic washer is highly recommended.)
6. Pipette 100 μL of diluted streptavidin-HRP conjugate into each well.
7. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
8. Wash the wells again in the same manner as in step 5.
9. Pipette 100 μL of TMB substrate into each well at timed intervals.
10. Incubate on a plate shaker for 10-15 minutes at room temperature.
11. Pipette 50 μL of stop solution into each well at the same timed intervals as in step 9.
12. Read the plate on a microwell plate reader at 450 nm within 20 minutes after the addition of the stop solution.

CALCULATIONS
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4 or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown sample duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 100 ng/mL, then dilute it with assay buffer at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.073</td>
<td>0.070</td>
<td>0.072</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0.102</td>
<td>0.100</td>
<td>0.101</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>0.290</td>
<td>0.293</td>
<td>0.292</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>0.620</td>
<td>0.630</td>
<td>0.625</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>1.140</td>
<td>1.086</td>
<td>1.113</td>
<td>20</td>
</tr>
<tr>
<td>F</td>
<td>1.947</td>
<td>1.919</td>
<td>1.933</td>
<td>50</td>
</tr>
<tr>
<td>G</td>
<td>2.518</td>
<td>2.514</td>
<td>2.516</td>
<td>100</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.275</td>
<td>0.273</td>
<td>0.274</td>
<td>4.22</td>
</tr>
</tbody>
</table>

TYPICAL CALIBRATOR CURVE
Sample curve only. Do not use to calculate results.
PERFORMANCE CHARACTERISTICS

SENSITIVITY
The limit of detection (LoD) for leptin is 0.50 ng/mL, as determined by use of a NCCLS protocol and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 82 blank determinations; LoB=0.42 ng/mL.

SPECIFICITY
The following substances were tested at 1000 ng/mL and exhibited no cross-reactivity: Mouse leptin, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, GM-CSF, CSF, and EGF.

INTRA-ASSAY PRECISION
Four serum samples were assayed twenty times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.45</td>
<td>0.09</td>
<td>3.7</td>
</tr>
<tr>
<td>2</td>
<td>7.54</td>
<td>0.34</td>
<td>4.3</td>
</tr>
<tr>
<td>3</td>
<td>11.87</td>
<td>0.84</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>27.51</td>
<td>1.37</td>
<td>5.0</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION
Four samples were assayed ten times over a period of ten days. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.71</td>
<td>0.16</td>
<td>5.9</td>
</tr>
<tr>
<td>2</td>
<td>8.24</td>
<td>0.48</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>12.01</td>
<td>0.82</td>
<td>6.8</td>
</tr>
<tr>
<td>4</td>
<td>24.36</td>
<td>1.45</td>
<td>5.8</td>
</tr>
</tbody>
</table>

RECOVERY
Spiked samples were prepared by adding defined amounts of leptin to three serum samples. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed</th>
<th>Expected</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Unspiked</td>
<td>3.89</td>
<td>6.95</td>
<td>90.4</td>
</tr>
<tr>
<td></td>
<td>6.28</td>
<td>11.95</td>
<td>91.9</td>
</tr>
<tr>
<td></td>
<td>10.98</td>
<td>25.43</td>
<td>94.4</td>
</tr>
<tr>
<td>2 Unspiked</td>
<td>7.69</td>
<td>8.95</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>8.82</td>
<td>15.03</td>
<td>107.7</td>
</tr>
<tr>
<td></td>
<td>30.32</td>
<td>30.06</td>
<td>104.7</td>
</tr>
<tr>
<td>3 Unspiked</td>
<td>11.81</td>
<td>15.81</td>
<td>99.4</td>
</tr>
<tr>
<td></td>
<td>15.71</td>
<td>24.41</td>
<td>104.1</td>
</tr>
<tr>
<td></td>
<td>41.18</td>
<td>41.07</td>
<td>100.3</td>
</tr>
</tbody>
</table>
LINEARITY
Three serum samples were serially diluted with leptin assay buffer. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed</th>
<th>Expected</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>1.42</td>
<td>1.52</td>
<td>93.4</td>
</tr>
<tr>
<td>1:4</td>
<td>0.71</td>
<td>0.76</td>
<td>93.4</td>
</tr>
<tr>
<td>1:8</td>
<td>0.35</td>
<td>0.38</td>
<td>92.1</td>
</tr>
<tr>
<td>2:1</td>
<td>11.27</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2:1.2</td>
<td>5.93</td>
<td>5.64</td>
<td>105.1</td>
</tr>
<tr>
<td>2:1.4</td>
<td>3.05</td>
<td>2.82</td>
<td>108.2</td>
</tr>
<tr>
<td>2:1.8</td>
<td>1.35</td>
<td>1.41</td>
<td>95.7</td>
</tr>
<tr>
<td>3:1</td>
<td>27.91</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3:1.2</td>
<td>14.91</td>
<td>13.96</td>
<td>106.8</td>
</tr>
<tr>
<td>3:1.4</td>
<td>6.74</td>
<td>6.98</td>
<td>96.6</td>
</tr>
<tr>
<td>3:1.8</td>
<td>3.29</td>
<td>3.49</td>
<td>94.3</td>
</tr>
</tbody>
</table>

COMPARATIVE STUDY
This Leptin ELISA was compared against a leading competitor’s Leptin EIA kit (Kit X). Thirty-eight serum samples ranging from 1.05-75.62 ng/mL were assayed with both kits, yielding the following results:
Regression: Kit X=0.9644 (11-LEPHU-E01) + 1.5489
r=0.98
Kit X Mean: 21.13
11-LEPHU-E01 Mean: 20.30

REFERENCES
APPENDIX 2

Prediktion av sjukdomsförloppet och dess relation till samsjuklighet vid symptomatisk knäartros - En studie av artrosfenotyper och deras biomarkörer

Bakgrund och syfte
Studier har visat att vissa personer med smärta i knäna utvecklar knäartros. Tidigare ansågs artros främst bero på slitage av ledbroset. Studier har dock på senare år visat att artros kan bero på andra saker, man har t.ex sett samband mellan artros, fetma, diabetes, hjärt-kärlsjukdomar, ämnesomsättningsrubbningar och utbredd smärta. Det gemensamma för dessa tillstånd är att man har en låg grad av inflammation. Man vet dock inte om inflammationen startar sjukdomarna eller om det är en konsekvens av dem. För att kunna studera dessa samband mer noggrant behöver vi följa personer med knäsmärta under en längre tid och med täta uppföljningar.

Förfrågan om deltagande
Vi vänder oss till dig som har besvär med smärta i knäna.

Hur går studien till?
Studien kommer att innebära att du kallas till en undersökning av dina knän en gång om året under 5 år. Röntgen och blodprover kommer också att göras en gång om året. Vid undersökningsställfällena kommer följande att utföras:

- Du kommer att få svara på ett frågeformulär med frågor bl.a. om smärta och smartupplevelse, samt livsstilsfaktorer (kost, rökning, alkoholvanor, fysisk aktivitet). Där finns även frågor kring dina knäbesvär, eventuella handbesvär samt allmän hälsa.
- Du får lämna blodprover för analys av ditt blodsocker, blodfetter samt för att se om du har en inflammation i kroppen.
- Du lämnar även blodprover som kommer att sparas i en biobank, för senare analys av specifika inflammations- samt broskmarkörer. Prov som sparas utgör en så kallad biobank. Biobankslagen säger att du som patient eller provgivare ska informeras och ge ditt samtycke till att prov sparas och för vilka ändamål de får användas. Dina blodprover kommer att sparas till dess de är analyserade och studien är slutförd. Proverna är aidentifierade i biobanken och kan endast identifieras av den person som
är ansvarig för studien. Har du frågor kring biobanken så kan du kontakta någon av de som är ansvariga, se sist i dokumentet.

Vilka är riskerna?
Det finns inga risker med att delta. Blodproverna och undersökningen av knäna kommer att utföras av utbildad personal. Röntgenundersökningen av dina knän bedöms ej medföra några risker.

Finns det några fördelar?
Du kommer att få ta del av dina resultat från samtliga tester.

Hantering av data och sekretess

Hur får jag information om studiens resultat?
Kunskapen från studieresultatet kommer att resultera i vetenskapliga artiklar, som kommer att skickas in till internationella reumatologiska tidskrifter samt till vetenskapliga kongresser. Alla resultat kommer att redovisas i grupp. Resultaten kommer även att delges personal på vårcentraler vid personalföreläsning samt föreläsningar för patientföreningar och kommer därmed att komma patienter till gagn.

Försäkring, ersättning
Försäkring för försökspersoner gäller, ingen ersättning för förlorad arbetsinkomst eller andra utgifter kopplade till projektet kommer att kunna utbetalas.

Frivillighet
Deltagande i forskningsprojekt är frivilligt och du kan när som helst, utan särskild förklaring, avbryta ditt deltagande. Du har rätt att begära att insamlad data och proverna förstörs eller märks så att de inte längre är möjliga att spåra dem till dig. Om du väljer att inte delta i studien eller avbryter studien kommer detta inte att påverka din behandling/omhändertagande.
FoU Spenshult, som är huvudman för studien lyder under personuppgiftslagen enligt vilken du har rätt att återkalla lämnat samtycke till att dina personuppgifter används i en studie. Lagen föreskriver också att du varje kalenderår kan få kostnadsfri information om vilka av dina personuppgifter som behandlas. Du kan även begära rättelse av personuppgifter. För begäran om rättelse eller information om personuppgifter kontakta: Maria Andersson (se nedan)

**Ansvariga**

Kontaktperson och projektledare:
Maria Andersson, BMA, Forskare vid FoU Spenshult
E-post: maria.andersson@spenshult.se, 0735-187043

Stefan Bergman
Leg läkare,
Forskningschef vid FoU Spenshult
Telefon 0735-187040 (sekr)

Ann Bremander
Leg sjukgymnast,
Bitr. forskningschef vid FoU Spenshult
Fanny Elmberg