Glucose and its association with metabolic factors and biomarkers in patients experiencing symptomatic knee osteoarthritis

A cross-sectional study

Bachelor’s thesis 15 credits in Exercise Biomedicine

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Frida Olsson
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## List of abbreviations and acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>BIA</td>
<td>Bioelectrical impedance analysis</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Hemoglobin A1c (glycated hemoglobin)</td>
</tr>
<tr>
<td>HCL</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High-sensitive CRP</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>ILs</td>
<td>Interleukins (group of cytokines)</td>
</tr>
<tr>
<td>KOA</td>
<td>Knee osteoarthritis</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic syndrome</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>VFA</td>
<td>Visceral fat area</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetramethylbenzidine</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor -α</td>
</tr>
</tbody>
</table>
Abstract

Background
Osteoarthritis (OA) is a long-term chronic disease that affects the joints and creates stiffness, pain and impaired movement. Knee osteoarthritis (KOA) is the most common form of OA and affects all tissues of the joint, including bone, muscles, synovia, and cartilage. Previously, OA was accepted as only an age- or mechanical stress-related degenerative joint disease, but more recent studies suggest that OA is a heterogenous disease including inflammatory, hormonal and metabolic factors such as abdominal obesity (visceral fat), lipids (cholesterol, HDL, LDL and triglycerides) and glucose.

Aim
The aim was to investigate the association of metabolic factors including fasting blood glucose, HbA1c, triglycerides, cholesterol, LDL, HDL, visceral fat, CRP and radiographic KOA in patients with symptomatic KOA.

Methods
Data from 91 patients was acquired in the ages 30 – 63 experiencing symptomatic KOA. All subjects where divided into two groups depending on their level of fasting glucose, high versus low. Group I (n=26) had high glucose levels ≥5,6 mg/L and group II (n=65) had low glucose levels <5,6 mg/L. Levels of HbA1c, lipids, visceral fat, CRP and radiographic KOA were then compared between the groups. Levels of fasting glucose, HbA1c and lipids (triglycerides, cholesterol, LDL, HDL) were analyzed by an accredited laboratory at the hospital of Halmstad by the department for labmedicine. CRP levels < 1 mg/L were manually analyzed with the sandwich ELISA method (enzyme-linked immunosorbent assay), which measures high-sensitive CRP (hsCRP) in serum. Visceral fat area was measured through bioelectrical impedance analysis (BIA) with InBody 770 and radiographs of the knees to obtain information about OA.

Results
There was a significant difference between the two groups in HbA1c, triglycerides, cholesterol and LDL p<0,05. Group I with high fasting glucose levels showed higher significant values of HbA1c, triglycerides, cholesterol and LDL than group II with low fasting glucose levels. 23% of all subjects met the requirement for metabolic syndrome according to IDF.

Conclusion
The findings in this study is in line with previous research and suggest that high glucose levels are associated with elevation of other metabolic factors in patients with knee osteoarthritis. However, there are several other interacting factors beyond the scope of this study, which may explain causalities. According to the findings in this study and previous research, obesity and metabolic syndrome could explain some of the connections between metabolic factors and knee osteoarthritis. Thus, further research is necessary to understand how all these metabolic factors are associated with osteoarthritis and obtain deeper knowledge about the pathogenesis and pathophysiology of the disease.

Keywords
Osteoarthritis, metabolic syndrome, HDL, LDL, glucose, HbA1c, CRP, visceral fat, low-grade inflammation
Abstract

Bakgrund

Artros är en långvarig kronisk sjukdom som påverkar lederna och skapar styvhet, smärtor och nedsatt rörelse. Knäartros är den vanligaste formen av artros och påverkar alla vävnader i leden, inklusive ben, muskler, synovialhinnan och brosk. Tidigare accepterades artros som enbart en ålders- eller mekanisk stressrelaterad degenerativ ledsjukdom, men senare studier tyder på att artros är en heterogen sjukdom som inkluderar inflammatoriska, hormonella och metabola faktorer som bukfetma (visceralt fett), lipider (kolesterol, HDL, LDL och triglycerider) och glukos.

Syfte

Syftet med studien var att undersöka sambandet mellan metabola faktorer inklusive fasteglukos, HbA1c, triglycerider, kolesterol, LDL, HDL, visceralt fett, CRP och radiografisk knäartros hos patienter med symptomatisk knäartros.

Metod

Data samlades in från 91 patienter i åldrarna 30-63, som upplevde symptomatisk knäartros. Alla patienter delades upp i två grupper beroende på deras nivå av fasteglukos, högt och lågt. Grupp I (n = 26) hade höga glukosnivåer ≥5,6 mg / L och grupp II (n = 65) hade låga glukosnivåer <5,6 mg / L. Nivåer av HbA1c, lipider, visceralt fett, CRP och radiografisk knäartros jämfördes sedan mellan grupperna. Nivåer av fasteglukos, HbA1c och lipider (triglycerider, kolesterol, LDL, HDL) analyserades av ett ackrediterat laboratorium på Halmstad sjukhus på avdelningen för labmedicine. CRP-nivåer <1 mg / L analyserades manuellt med sandwich-ELISA-metoden som mäter högsensitiv CRP (hs-CRP) i serum. Visceral fetmassa mättes genom bioelektrisk impedansanalys med InBody 770 och röntgenbilder av knäna för att erhålla information om knäartros.

Resultat

Det var en signifikant skillnad mellan de två grupperna i nivåer av HbA1c, triglycerider, kolesterol och LDL p <0,05. Grupp I med höga glukosnivåer visade högre signifikanta värden av HbA1c, triglycerider, kolesterol och LDL än grupp II med låga glukosnivåer. 23% av alla patienter uppfyllde kraven för metabolt syndrom enligt IDF.

Slutsats

Resultaten i denna studie ligger i linje med tidigare forskning och föreslår att höga glukosnivåer är förknippade med höjning av andra metaboliska faktorer hos patienter med knäartros. Det finns emellertid flera andra interagerande faktorer som ligger utanför ramen för denna studie, vilket kan förklara kausalitet. Enligt resultaten i denna studie och tidigare forskning kan fetma och metabolt syndrom förklara några av sambanden mellan metabola faktorer och knäartros. Således krävs ytterligare forskning för att förstå hur alla dessa metabola faktorer är associerade med artros och få en djupare kunskap om sjukdomens patogenes och patofysiologi.
Background
Osteoarthritis (OA) is a long-term chronic disease that affects the joints and creates stiffness, pain and impaired movement. The disease is becoming an increasingly common public health disorder with a major socio-economic impact due to disability. OA is the most common rheumatic joint disease in Sweden, affecting 25% of the population over 45 years old. However, it can be debuted already in the ages 20-30. The incidence of OA is expected to increase as the population is getting older and due to an increase in obesity among the population (Englund & Turkiewicz, 2014).

Over the past 13 years, people over 45 years old that searched medical care for OA where divided as following; knee osteoarthritis (KOA) 12%, hip 5% and hand 3% according to data from Region Skåne (Englund & Turkiewicz, 2014). A population-based cohort study through Skåne Health Care Register data linked with data from the Swedish Social Insurance Agency, was made between 1998-2009 on Skåne county residents diagnosed with KOA during this time period. They found that the mean number of sick days in patients with KOA was 87 annually and 57 for the general population. Of all sick days in the society patients with KOA stands for 2% and compared to the general population, patients with KOA also have about 40-50% increased risk of disability pension (Hubertsson, et al., 2013).

The pathogenesis of OA is complex, and it is important to investigate the underlying factors in order to prevent and treat the disease. Before OA was accepted as only an age- or mechanical stress-related degenerative joint disease, but more recent studies suggest that OA is a heterogenous disease including inflammatory, hormonal and metabolic factors (Belluzzi, et al., 2017). These findings include impaired and dysregulated lipid- and glucose metabolism, resulting in elevated levels that affects different tissues of the joint (Laigullion, et al., 2015) (Masuko, et al., 2009).

Pathophysiology of knee osteoarthritis
Knee osteoarthritis is the most common form of OA according to Dell’Isola et al. (2016) and more recent studies have found that it affects all tissues of the joint, including bone, muscles, synovia, and cartilage. The disease is characterized by joint degeneration including destruction of articular cartilage, bone remodeling, synovial inflammation and impaired function of the joint (de Munter, et al., 2016). In OA, the tissue homeostasis of
Articular cartilage and subchondral bone is changed due to several external and internal factors. Among these factors are pro-inflammatory mediators (including cytokines), proteolytic enzymes, lipids and glucose, that affects bone metabolism (Ashkavand, et al., 2013). Synovitis (inflammation of the synovial membrane) in OA is associated with cartilage breakdown. Homeostasis of cartilage matrix degradation and repair is changed due to proinflammatory cytokines, nitric oxide, neuropeptides and prostaglandins produced by the synovium, which leads to cartilage degradation by excess production of proteolytic enzymes. The synovitis is then further aggravated due to cartilage breakdown, creating an vicious circle (Sellam & Berenbaum, 2010). Figure 1 shows a schematic picture of the pathophysiology of knee osteoarthritis together with the proinflammatory mediators and immune cells involved.

Assessment of KOA is commonly executed by radiography which shows the progression of joint space narrowing (JSN). The loss of articular cartilage in KOA generally results in JSN which is clearly shown by radiographs (Wing, et al., 2008).

Figure 1. Pathophysiology of knee osteoarthritis (Sellam & Berenbaum, 2010)

Metabolic factors and biomarkers

More than 50% of the swedish population are overweight, of which 10% are obese. This means a 100% increase since 1980’s (Folkhälsomyndigheten, 2018). Obesity is accompanied by several metabolic changes in the body and among these are hypertension, hyperlipidemia and impaired glucose control (Norberg & Danielsson, 2009). Low physical activity and high intake in sugar and fat will lead to a positive energy balance that may lead
to obesity and cause impaired glucose tolerance, insulin resistance, hypertension, atherogenic dyslipidemia and intra-abdominal adiposity (accumulation of visceral fat), which are all cardiometabolic risk factors (Srivastava, 2012). Several studies suggest that excess accumulation of visceral fat induced by feeding and low physical activity is associated with elevated levels of HbA1c, LDL, insulin resistance and elevated circulation of inflammatory mediators (Bawadi, et al., 2016).

Visceral fat function as metabolic active tissue and is associated with low-grade systemic inflammations in terms of pro-inflammatory cytokines that it produces and secretes into the bloodstream. Pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α), are produced and transported by the bloodstream to the liver where they activate the expression of c-reactive protein (CRP), an acute phase reactant that can be measured through blood samples (Srivastava, 2012). Low-grade inflammation is a chronic inflammatory response which has a strong relationship with cardiometabolic diseases, including metabolic syndrome, and has recently been referred to as a metabolic inflammation (José Israel, et al., 2014). Several studies suggest that low-grade inflammation may lead to diabetes mellitus type II, insulin resistance, dyslipidemia, hypertension and several inflammatory initiated diseases such as osteoarthritis, and has increased during the last decade. Which molecular events and cellular mechanisms that play a role in metabolic inflammation are still researched and further discussed (José Israel, et al., 2014).

**Metabolic syndrome**

Metabolic syndrome (MetS) is not a disease but a collective term for several risk factors that may lead to cardiovascular diseases, such as stroke and heart attack. These factors include visceral obesity, dyslipidemia (raised triglycerides and cholesterol), hyperglycemia (raised fasting plasma glucose) diabetes and hypertension (Alberti, et al., 2005). The pathogenesis of MetS is complex and include an interaction between genetics and lifestyle. Obesity, especially abdominal obesity (visceral fat) together with insulin resistance in adipose tissue, liver and skeletal muscle tissue, have a central role in the pathogenesis. Elevated levels of LDL and triglycerides and low HDL levels are other important components of MetS. Fatty liver, hypertension and systemic inflammation are also common among patients with MetS (Hellenius & Rauramaa, 2007). Definition of metabolic syndrome is according to International Diabetes Federation (IDF) a person who
has central obesity (defined as waist circumference with ethnicity specific values) plus any two of following factors: (Table 1.)

**Table 1. Metabolic risk factors**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raised triglycerides</strong></td>
<td>≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality</td>
</tr>
<tr>
<td><strong>Reduced HDL cholesterol</strong></td>
<td>&lt; 40 mg/dL (1.03 mmol/L) in males &lt; 50 mg/dL (1.29 mmol/L) in females</td>
</tr>
<tr>
<td><strong>Raised blood pressure</strong></td>
<td>systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension</td>
</tr>
<tr>
<td><strong>Raised fasting plasma glucose</strong></td>
<td>(FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes</td>
</tr>
</tbody>
</table>

© International Diabetes Federation, 2006

**Type II diabetes mellitus and osteoarthritis**

Type II diabetes mellitus (T2DM) is characterized by impaired glucose tolerance, insulin resistance and relative insulin deficiency. High blood-glucose over time (hyperglycemia), increased endogenous production of glucose, decreased peripheral glucose-utilization and β-cell dysfunction are features of T2DM. Elevated free fatty acids in the bloodstream due to increased lipolysis contribute to further aggravate these features (DeFronzo, et al., 2015). T2DM is considered to be one of the cardiometabolic diseases and is part of the metabolic syndrome which increases risk of an early death. T2DM is increasing worldwide and there are strong evidences that the disease correlates with physical inactivity and poor food habits. Low-grade inflammation may also play a role in development of T2DM by inducing insulin resistance and may be a link between obesity and T2DM. Some researchers suggest there might be a genetic disposition for the disease but most likely the result of environmental, behavioral and genetic factors together (Tuomilehto, et al., 2001).

As mentioned earlier, several recent studies suggest that low-grade systemic inflammation may play a role in the development and progression of OA, which have a strong
relationship with cardio-metabolic factors. Epidemiological studies have more recently found an eventual connection between OA and type II diabetes mellitus, as one of many cardio-metabolic factors (Laigullion, et al., 2015). Interleukin 1β (IL-1β), which is a cytokine protein produced by macrophages and part of the inflammatory response, is increased in both T2DM and OA. Other low-grade inflammatory mediators such as IL-6, IL-8 and reactive oxygen species (ROS) are also increased in both diseases. Except T2DM correlation with inflammatory mediators involved in OA, researchers suggest that hyperglycemia affects OA cartilage degradation. Patients with untreated type II diabetes mellitus have hyperglycemia, which affect the joint environment by elevating glucose-levels in extracellular matrix (EM). Excessive EM glucose-levels leads to increased intracellular glucose concentrations in chondrocytes, which induce cellular stress through several biochemical pathways. ROS-production also increases due to elevated intracellular glucose-levels and may affect cartilage homeostasis (Laigullion, et al., 2015). Imbalance between ROS and reactive nitrogen species (RNS) induce oxidative stress (Modun, et al., 2014). The main nitrogen active species is nitric oxide (NO), which has a pro-inflammatory and pro-degradative effects on chondrocytes (Laigullion, et al., 2015). NO release is elevated by inflammatory mediators such as IL-β and TNF-α and correlates with activity of protease enzymes, metalloproteases, that are involved in inflammatory-induced cartilage catabolism (Murrell, et al., 1995).

Detection of hyperglycemia in T2DM patients is made by measuring fasting-glucose in blood plasma. When fasting, α-cells in the pancreas releases glucagon, which is a hormone stimulating gluconeogenesis in the liver (stored glycogen converts to glucose). Glucose is then released into the bloodstream raising the plasma-glucose levels. In healthy individuals, insulin is released in response through β-cell stimulation in the pancreas, which are sensitive to high glucose levels. Insulin delivers glucose into insulin-dependent tissue: muscle cells, adipose tissue and liver. In T2DM patients, the insulin production is not sufficient and/or absorption of glucose into insulin-dependent tissue is impaired, resulting in high fasting-glucose plasma levels (Roden, et al., 2017).

Glycated hemoglobin (HbA1c), is a form of hemoglobin that reflects the average blood glucose levels over an approximately three-month time period, which is the lifespan of erythrocytes. Glucose molecules binds to HbA1c, which is a protein on the surface of erythrocytes. The HbA1c test measures the amount of glucose bound to hemoglobin in
erythrocytes, where higher blood glucose levels lead to more glucose bound to hemoglobin and a higher HbA1c result (Davis, 2016). In patients with diabetes mellitus, the HbA1c test is useful to determine glycemic control over time and is used routinely. Some medical doctors even suggest the HbA1c test for diagnosis of diabetes mellitus and as a screening test for people at high risk for the disease (World Health Organization, 2011). Reference interval for HbA1c is according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC):

- < 50 years old: 27 - 42 mmol/mol
- ≥ 50 years old: 31 - 46 mmol/mol

**Lipids and osteoarthritis**

Triglycerides is the main part of lipids (fats) in food and adipose tissue. Cholesterol are structural lipids that is both part of the cell membrane along with phospholipids, and a part of lipoprotein that function as transport for lipids in the bloodstream. Cholesterol are supplied by food ingestion or by endogenous synthesis mainly in the liver. When discussing cholesterol, we often refer to low-density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL “the bad cholesterol” is the particle with most cholesterol (45%), which has a main task to provide cholesterol to the cells. HDL “the good cholesterol” on the other hand consists of less cholesterol (22%) and function mainly as a carrier of cholesterol from the cells to the liver for breakdown. An unhealthy diet rich in fats, mostly saturated and trans fats, and low physical activity leads to elevated LDL-cholesterol and decreased HDL-cholesterol (Abrahamsson, et al., 2006). The imbalance results in excessive LDL-cholesterol in the bloodstream, which ends up in macrophages and other immune cells, vessel walls, liver and spleen. Accumulation of cholesterol in immune cells promotes inflammatory response (Tall & Yvan-Charvet, 2015).

Association between hypercholesteremia and OA has been found in several recent studies. Hypercholesteremia, also called high cholesterol, refers to increased levels of LDL-cholesterol and decreased levels of HDL-cholesterol. Cholesterol-induced inflammatory response contributes to low-grade systemic inflammation in the body and promotes several metabolic diseases, OA included (Tall & Yvan-Charvet, 2015). Macrophages in the synovial lining of the joint induce synovial inflammation in OA and researchers have found an association between markers for systemic inflammation and synovial inflammation.
(Pearle, et al., 2004). Recent studies of experimental and human OA also found that high levels of LDL-cholesterol contribute to cartilage destruction (de Munter, et al., 2016).

In healthy individuals’ total cholesterol should be < 7,8 mmol/L and LDL < 5,3 mmol/L. Reference interval for HDL according to Labmedicine in Halland county is 0,8 – 2,7 mmol/L. “High total cholesterol” is not what’s important to investigate, but the ratio cholesterol/LDL or LDL/HDL due to their different characteristics and physiological function in the body. LDL is the “bad” atherogenic cholesterol and HDL is the “good” non-atherogenic cholesterol (Wahlberg, 2008).

C-reactive protein

C-reactive protein (CRP) is a protein found in blood plasma and serum in humans. It is a pentameric ring-shaped protein whose levels rises in response to inflammation and is thereby classified as an acute-phase reactant and biomarker for inflammation (Black, et al., 2004). CRP is synthesized by hepatocytes in the liver and is normally present at less than 3 mg/L in blood plasma and serum in healthy individuals but can rise as much as 1000-fold due to an acute inflammatory stimulus (Macy, et al., 1997). CRP synthesis is triggered by cytokines and is largely regulated by the proinflammatory cytokine IL-6, which is produced and released into the bloodstream by macrophages and adipocytes (Anon., 1999). CRP can be measured using enzyme-linked immunosorbent assay as described in the method.

Aim

Though all these metabolic factors and biomarkers including blood glucose, HbA1c, LDL, visceral fat and CRP may have a role in the development of knee osteoarthritis, it is important to study the association of these in patients with early stage KOA referred as symptomatic KOA. The understanding of changes in an early stage of the disease could lead to better treatment that will reverse or slow down progression (Man & Mologhianu, 2014). The gained knowledge could even lead to prevention of osteoarthritis.

Research question

Is there a difference in levels of lipids, C-reactive protein, visceral fat, HbA1c and radiographic knee osteoarthritis in subjects with high verses low fasting glucose levels?
Methods

This is an empirical study with cross-sectional design, in collaboration with an ongoing extensive prospective cohort “Detection and prediction of disease course in symptomatic knee osteoarthritis” by FoU Spenshult in Halland county.

The subjects were divided into two groups depending on high and low fasting glucose levels. The split was made in quartiles, in SPSS statistics 17 software, where the upper quartile (25% of all subjects) formed group I and the rest formed group II. The glucose level of division was at 5.6 mmol/L, which is IDF definition of metabolic syndrome. Group I: n=26 had glucose level 5.6 mmol/L or higher and group II: n=65 had glucose levels lower than 5.6 mmol/L. Levels of lipids, C-reactive protein, visceral fat, HbA1c and radiographic knee osteoarthritis were compared between the two groups.

Subjects

91 subjects were included in this study between the ages 30 – 60, experiencing symptomatic knee osteoarthritis and searched medical care at primary health care centers in southern part of Halland, Sweden. Subjects with signs of inflammatory rheumatic disease or knee trauma were excluded for this study. The subjects were recruited through advertising in different newspapers of Halland county (Hallands Nyheter, Hallandsposten, 7dagar and Laholms tidning).

Clinical examinations

Information about visceral fat area (VFA) were conducted through bioelectrical impedance analysis (BIA) of body composition with InBody 770 (Figure 2). The device has 4 built in electrodes, two at the base where the feet are placed and in two handles connected to the device which are hold by the hands. The principle of the method is that electrical current with varying frequencies are sent from the electrodes, passing through the body and measuring impedance (electrical resistance) in different body tissues. Different frequencies are used due to their penetration ability. Some frequencies measure only extracellular fluid while others may pass the cell membrane to measure intracellular fluid and total body fluid. InBody 720 uses 6 different frequencies to measure each segment and get a total of 30 different impedance values, providing precise and accurate information about body
composition (InBody USA, 2017). This is a highly valid and reliable method for research and population samples if following correct standardization (von Hurst, et al., 2016).

Standardization of measurement with InBody is very important to obtain reliability of the method. Ideally, the measurement should be done early in the day on an empty stomach and with as little clothing as possible. Food and beverages in the stomach and clothes can give a misleading result. Exercising before a measurement should also be avoided as this may temporarily change the body composition. It is important that the standardization is the same for measurements 1, 2, 3 ... etc. in order to provide reliable results (InBody USA, 2017).

The BIA method was validated against Air displacement plethysmography (ADP) and dual-energy X-ray absorptiometry (DXA), and reliability tested in a study by (von Hurst, et al., 2016) which showed excellent relative agreement to the estimated true value ($\rho = 0.97 \ (0.96, 0.98)$ in measuring body fat.

The measurement was proceeded by the subjects one by one. Clothes were discarded before the measurement and the subject stepped onto the device with their feet placed on the electrodes. Information about age and height were entered on the device. The subject was told to hold the handles of the device and the measurement could commence. Time for each measurement was about one minute and a body composition result sheet was obtained (figure 2). VFA was the collected information for this study and should not be higher than 100 cm$^2$ in healthy individuals.

![InBody 770 and body composition result sheet](InBody USA, 2017)
Radiographic assessment

Radiographs of both knees by weight-bearing X-rays were executed on both knees in a standing position with equal load on both feet. The radiographic assessment was performed by a medical doctor at Halmstad hospital in Halland county and was executed at three angles: frontal, vertical and skyline-view of patello-femoral joint demonstrating joint space narrowing.

Biomarkers

Blood samples were obtained for analyses of fasting-glucose, glycated hemoglobin (HbA1c), lipids (triglycerides, cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL)), to get information about lipids and blood-sugar levels, and also analyses of C-reactive protein (CRP) which is a marker for inflammation. CRP > 1 mg/L, P-glucose, HbA1c, triglycerides, cholesterol, LDL and HDL were analyzed by the department for laboratory medicine at the hospital of Halmstad. All blood samples were saved in a biobank for future studies.

CRP < 1,0 mg/L, high-sensitive CRP must be manually analyzed with ELISA, which is designed to measure small concentrations with high sensitivity (Abnova corporation, 2018).

Lipids and glucose analysis

All lipids, glucose, HbA1c and CRP > 1mg/L were analyzed by an accredited laboratory at the hospital of Halmstad (the department for clinical chemistry).

CRP > 1,0 mg/L was analyzed using turbidimetry with Cobas 8000 (Roche). Results were presented in mg/L.

P-glucose was measured using photometry with Hemocue Glucose 201 DM RT (HemoCue). Results were presented in mmol/L.

HbA1c was analyzed using liquid chromatography with TOSOH G8. Results were presented in mmol/mol.

All lipids (triglycerides, cholesterol, LDL and HDL) was analyzed using photometry with Cobas 8000 (Roche). Results were presented in mmol/L.
Table 2. Reference intervals according to laboratory medicine in Halland county

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>0 – 4,9 mg/L</td>
</tr>
<tr>
<td>p-glucose</td>
<td>4,2 – 6,3 mmol/L</td>
</tr>
<tr>
<td>HbA1c (IFCC)</td>
<td>&lt; 50 years: 27 – 42 mmol/mol, ≥ 50 years: 31 – 46 mmol/mol</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0,5 – 2,6 mmol/L</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>31 – 50 years: 3,3 – 6,9 mmol/L, &gt;50 years: 3,9 – 7,8 mmol/L</td>
</tr>
<tr>
<td>LDL</td>
<td>31 – 50 years: 1,4 – 4,7 mmol/L, &gt;50 years: 2,0 – 5,3 mmol/L</td>
</tr>
<tr>
<td>HDL</td>
<td>women: 1,0 – 2,7 mmol/L, men: 0,8 – 2,1 mmol/L</td>
</tr>
</tbody>
</table>

CRP ELISA analysis

CRP levels > 1 mg/L was analyzed by the department for laboratory medicine at the hospital of Halmstad, using the turbidimetry method of a plasma sample. CRP levels < 1 mg/L must be manually analyzed with the ELISA method, which measures high-sensitive CRP (hsCRP) in serum. The hsCRP ELISA kit used was a sandwich method which is based on the principle of solid phase enzyme-linked immunosorbent assay and quantification of protein between two layers of antibodies. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic epitope on the on the CRP molecule, which is capable of binding to the antibody. Coated on the microtiter wells were the capture antibodies, mouse monoclonal anti-CRP antibodies, that recognizes the specific epitope on the CRP molecule (Abnova corporation, 2018).
Material

*Table 3. List of components*

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-Coated Wells: Microtiter wells coated with mouse monoclonal anti-CRP.</td>
<td>96 wells</td>
</tr>
<tr>
<td>Reference Standard Set: Contains 0, 0.005, 0.010, 0.025, 0.050 and 0.100 mg/L CRP in serum-based buffer with preservatives.</td>
<td>1 mL x 6</td>
</tr>
<tr>
<td>hsCRP Sample Diluent: Contains phosphate buffer-BSA solution with preservatives. 50</td>
<td>50 mL</td>
</tr>
<tr>
<td>CRP Enzyme Conjugate Reagent: Contains goat anti-CRP conjugated to horseradish peroxidase with preservatives.</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Reagent (One-Step)</td>
<td>11 mL</td>
</tr>
<tr>
<td>Stop Solution (1N HCl)</td>
<td>11 mL</td>
</tr>
</tbody>
</table>

*Received from:* (Abnova corporation, 2018)

Procedure

Serum samples were collected and stored at -80°C and before the CRP assay, the samples were thawed to room temperature. The serum was then added to the wells on the microtiter plate. Standard samples with known CRP concentrations were added to the first four wells to obtain a standard curve. A solution with detection goat anti-CRP antibodies and enzyme conjugate (horseradish peroxidase) was added to the wells resulting in the CRP molecules being sandwiched between the solid-phase and the enzyme-linked antibodies. The wells were washed with distilled water after 45 minutes of incubation in room temperature to remove unbound labelled antibodies. The reagent substance tetramethylbenzidine (TMB) was added and incubated for 20 minutes. TMB reacts with the enzyme-labeled antibodies and the color turns blue in the wells. In the last step a stop-solution was added (HCL), which stopped the reaction and changed the color to yellow. Absorbance of the resulting yellow colored samples were measured spectrophotometrically using a microtiter well reader (Tecan Sunrise, 2017). The absorbance is directly proportional to the concentration of CRP in the samples.
Though the hsCRP ELISA assay is manually performed, the need for rigorous standardization of the procedure is necessary. The CRP samples and different solutions are pipetted by hand and is the most critical part of the method. But space for small errors is given due to the human factor and by correctly executed pipetting the method remains highly reliable.

Figure 2. Principle of Sandwich ELISA

Statistics

Statistical analyses were performed using SPSS Statistics 17 software. All significant tests were 2-tailed and conducted at 0.05 significance level. To test if variables were normally distributed Shapiro-Wilks (<0.05) were used. The chi-square test was used to test differences between groups and the Mann-Whitney U test when comparing two groups for continuous variables, because some of the variables were not normally distributed.

Null-hypothesis: distribution of Hba1c, triglycerides, cholesterol, HDL, LDL, CRP and visceral fat area is the same across categories of glucose groups (Mann-Whitney U test >0.05).

Ethical and social considerations

All patients received information about the study and gave their informed consent (appendix I) and the study was performed in accordance with the Declaration of Helsinki. Ethical approval was obtained (EPN 2016-229-2017/253). Collected data was handled in
accordance with confidentiality requirements and personal data was handled carefully according to the personal data act. Participation in this study did not present any direct risks, side effects, pain or discomfort. Blood sampling and measure of body composition could be perceived as an integrity restriction. Participants measured body composition individually in their underwear, which could cause emotional discomfort. In case of discomfort or for any other reason, the participants were entitled to discontinue their participation in the study at any time.

About social considerations, this study could contribute to several benefits in a bigger perspective, such as reducing extensive social costs (especially healthcare and sick leave) due to osteoarthritis. The results will lead to a deeper understanding about the complex pathophysiology and pathogenesis of the disease, what factors may cause osteoarthritis and affects the progression. The obtained knowledge can lead to more effective treatment in an early stage and prevent or reverse progression. This in turn will reduce the burden on society and the health care system though individuals can work longer with less sick leave. Better understanding and treatment of the disease will also contribute to higher quality of life at the individual level.

Results

Descriptive characteristics
91 subjects participated in the study, 26 men and 65 women. Median value for age was 54 (30-63). 23% of all subjects met the requirement for metabolic syndrome according to IDF. All subjects where divided into two groups: group I (n=26, 65% women) with glucose level 5,6 mmol/L or higher, group II (n=65, 74% women) with glucose levels lower than 5,6 mmol/L. Median value for age in group I was 54 (36-61) and 53 (30-63) for group II. HbA1c, triglycerides, cholesterol and LDL reject the null-hypothesis p<0.05, where group I had significant higher levels of HbA1c, triglycerides, cholesterol and LDL than group II (table 4). Some of the subjects had levels outside the reference interval for the measured parameters (table 2): elevated cholesterol: n=3, elevated triglycerides: n=1, low HDL: n=1, elevated LDL: n=7, elevated CRP (>5): n=6, elevated glucose: n=2, VFA above 100 cm^2: n=39.
CRP measurements for all subjects can be viewed in *figure 3*, whereas you will find CRP measurements for group I in *figure 4*, and for group II in *figure 5*. Median value for CRP in group I = 1.25 (0.2-59.7) and for group II = 1.2 (0.2-10.5) (Table 4). An outlier with CRP level 59.7 mg/L (Table 4) was excluded in *figure 3 and 4*. The subject had a severe cold at the time for measurement and showed a significant lower result when measuring later on.

**Table 4. Characteristics of subjects with high (≥5,6 mmol/L) and low (<5,6 mmol/L) glucose levels**

<table>
<thead>
<tr>
<th></th>
<th>All Median (min-max)</th>
<th>Group I Glucose ≥ 5,6</th>
<th>Group II Glucose &lt; 5,6</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>91</td>
<td>26</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>54 (30-63)</td>
<td>54 (36-61)</td>
<td>53 (30-63)</td>
<td>0,471</td>
</tr>
<tr>
<td>Women % (n)</td>
<td>71 (65)</td>
<td>65 (17)</td>
<td>74 (48)</td>
<td>0,420</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>36,0 (28,0-54,0)</td>
<td>38,0 (31,0-54,0)</td>
<td>35 (28,0-44,0)</td>
<td><strong>0,003</strong></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5,3 (3,5-8,7)</td>
<td>5,8 (3,7-7,4)</td>
<td>5,2 (3,5-8,7)</td>
<td><strong>0,008</strong></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0,9 (0,4-5,0)</td>
<td>1,2 (0,4-2,2)</td>
<td>0,8 (0,4-5,0)</td>
<td><strong>0,002</strong></td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1,7 (0,9-2,9)</td>
<td>1,6 (0,9-2,8)</td>
<td>1,8 (1,1-2,9)</td>
<td>0,115</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3,5 (1,8-23,0)</td>
<td>4,1 (1,8-5,5)</td>
<td>3,3 (1,9-23,0)</td>
<td><strong>0,017</strong></td>
</tr>
<tr>
<td>P-CRP (mg/L)</td>
<td>1,2 (0,2-59,7)</td>
<td>1,25 (0,2-59,7)</td>
<td>1,2 (0,2-10,5)</td>
<td>0,100</td>
</tr>
<tr>
<td>P-glucose (mmol/L)</td>
<td>5,3 (4,2-9,4)</td>
<td>5,8 (5,6-9,4)</td>
<td>5,1 (4,2-5,5)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>VFA (cm²)</td>
<td>92,4 (7-291)</td>
<td>117,9 (52-291)</td>
<td>89,7 (7-238)</td>
<td>0,067</td>
</tr>
<tr>
<td>X-ray % (n)*</td>
<td>48 (41)</td>
<td>52,0 (13)</td>
<td>46,7 (28)</td>
<td>0,654</td>
</tr>
</tbody>
</table>

*Number of patients with radiographic knee osteoarthritis*
Figure 3. Dot plots of all CRP measurements (mg/L), n=91

Figure 4. Dot plots of CRP measurements for group I (mg/L), n=26
Discussion

Results
There was a significant difference between the two groups in HbA1c, triglycerides, cholesterol and LDL. Group I with glucose level 5.6 mmol/mol or higher had significant higher values of HbA1c, triglycerides, cholesterol and LDL than group II as predicted. The results are in line with previous research as high glucose levels, mostly induced by feeding, is associated with other cardiometabolic factors as mentioned earlier in research by Srivastava, 2012, Bawadi, et al., 2016 and Alberti, et al., 2005 among others. The reason why VFA and radiographic KOA did not show significant higher values and significant lower HDL values in group I may be due to the low number of subjects in group I (n=26). However, the results show tendencies of higher VFA values, radiographic KOA and lower HDL values in group I with higher glucose levels (table 4) which might be considered as a trend which is important to have in mind in future investigations within the area. Furthermore, the results showed higher values of HbA1c, cholesterol, triglycerides, LDL, VFA and CRP in some subjects than recommended for healthy individuals (outside reference interval) in the whole group. Few of these values were extreme outliers, such as CRP level 59.7 mg/L in group I. The subject with CRP level...
59.7 mg/L had a severe cold at the time for measurement and showed a significant lower result when measuring later on, thus the value was excluded in figure 3 and 4 due to a misleading picture of reality. CRP is an acute phase reactant which level rises due to inflammation or infection in the body. The aim of this study was to investigate the association between metabolic factors and biomarkers in patients with symptomatic KOA, including low-grade inflammation. CRP is a biomarker for low-grade inflammation but also a biomarker for infections. The patient with CRP level 59.7 mg/L was therefore excluded in the dot-plot charts to give a more accurate depiction.

According to Labmedicine in Halland county healthy individuals should have total cholesterol < 7.8 mmol/L and LDL < 5.3 mol/L. n=3 had elevated total cholesterol and n=1 had elevated LDL. As for total cholesterol it is important to understand that it includes both LDL “atherogenic bad cholesterol” and HDL “non-atherogenic good cholesterol”. This means that what’s important to evaluate is the ratio total cholesterol/LDL or HDL/LDL to determine what is healthy or unhealthy. Values for HDL in the whole group (table 4) were within reference interval except for n=1 which was too low, according to Labmedicine in Halland county. However, the mean value for HDL was lower in group I than group II (1.6 mmol/L and 1.8 mmol/L respectively). The highest and lowest value for HDL were also lower in group I than group II (table 4). Elevated levels of triglycerides and LDL and low HDL levels together with raised glucose levels agrees with the definition of metabolic syndrome (Hellenius & Rauramaa, 2007).

23% of all subjects met the requirement for metabolic syndrome according to IDF. When comparing reference values for metabolic syndrome, in terms of cholesterol, triglycerides, and glucose (table 1) and reference values for healthy adults according to Labmedicine in Halland county (table 2), there are differences. Values for cholesterol, triglycerides, and glucose in healthy adults according to Labmedicine in Halland county are higher than those for metabolic syndrome according to IDF. This means that the healthcare might not observe those patients in the risk zone or have metabolic syndrome with current “normal” values.

Obesity, elevated levels of lipids and glucose together with inflammation are risk factors that link osteoarthritis and MetS together which agrees with the results of this study and
several other recent studies including a systemic review by Hussain, et al., 2014. where they found that these factors increased risk for KOA.

**Methods**

All lipids, glucose, HbA1c and CRP > 1mg/L were analyzed by an accredited laboratory at the hospital of Halmstad (the department for clinical chemistry), and their methods are highly valid and reliable “gold standard”. As for measurement of body composition with InBody bioelectrical impedance analysis (BIA), it is very important to follow standardization for the test, which was difficult to achieve in this study. What is recommended according to InBody’s standardization is to measure in the morning before food and beverage ingestion, no physical exercising before or alcohol and tobacco use. This was difficult to achieve and control and should be considered when evaluating the results. The CRP-ELISA kit used to analyze hs-CRP was controlled by the department for laboratory medicine at the hospital of Halmstad before analysis at FoU Spenshult which showed accuracy. However, as the test was performed manually, preciseness when pipetting and the use of rigorous standardization of the procedure were of high importance to obtain correct result. When the analysis was performed, focus was on top and standardization was followed, and the procedure went through without any errors.

**Conclusion**

A quarter of the patients with symptomatic knee osteoarthritis, included in the study, had elevated glucose levels according to IDF. These also had increased levels of total cholesterol, LDL cholesterol, triglycerides, HbA1c, CRP, higher VFA and lower HDL. Although the results only showed significant differences in levels of total cholesterol, LDL, triglycerides and HbA1c between the two groups (high vs low glucose levels). The findings in this study is in line with previous research and suggest that high glucose levels are associated with elevation of other metabolic factors in patients with osteoarthritis. However, there are several other interacting factors beyond the scope of this study, which might could explain causalities. According to the findings in this study and previous research, obesity and metabolic syndrome could explain some of the connections between metabolic factors and knee osteoarthritis. Thus, further research is necessary to understand how all these metabolic factors are associated with osteoarthritis and obtain deeper knowledge about the pathogenesis and pathophysiology of the disease.
References


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Appendix I

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 ledbrosket. 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, ämnesomsättningssrubningar 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 undersökningsstillfällena kommer följande att utföras:

- Du kommer att få svara på ett frågeformulär med frågor bl.a. om smärta och smärtupplevelse, 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 avidentifierade i biobanken och kan endast

Sida 27(30)
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 knä 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 vårdcentraler 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
Jag har fått information om studien, dess syfte och fått möjlighet att ställa frågor. Jag deltar frivilligt i studien och kan när som helst avbryta min medverkan.

Ort och Datum:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Namnteckning
________________________________________________________

Namnförtydligande

Om du fyller i din E-post nedan så kommer vi att skicka resultaten på mätningarna

Epost:__________________________________________________________________
_______
I am a health worshiper with a burning interest in diet, health and physical exercise. My future research area will be in nutrition epidemiology, to be able to help people help themselves before it is too late. My goal is to raise people’s quality in life.