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Association between visceral fat, obesity and biomarkers for inflammation in subjects experiencing symptomatic knee pain

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Association between visceral fat, obesity and biomarkers for inflammation in subjects experiencing symptomatic knee pain

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Bachelor Thesis 15 credits in Exercise Biomedicine
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Abstract

Background

Osteoarthritis (OA) is a common, chronic disorder with a complex etiology including genetic and environmental factors. Metabolic triggered inflammation induced by obesity contributes to the inflammatory process and higher levels of pro-inflammatory cytokines and adipokines, which may play a role in OA pathophysiology. Obesity-related metabolic factors such as adipokines, contribute to OA development by inducing pro-inflammatory cytokines and degradative enzymes, leading to cartilage matrix damage and subchrondal bone remodeling.

Aim

The aim of this study was to examine the association between visceral fat, obesity and a biomarker for inflammation (C-reactive protein, CRP) in a group of people experiencing symptomatic knee pain.

Methods

Adults (n=89) of varying ages between 30-63 years, experiencing symptomatic knee pain or diagnosed knee OA were tested once. Levels of CRP were analyzed with the sandwich ELISA method (Enzyme-Linked ImmunoSorbent Assay). Visceral Fat Area (VFA) and Body Mass Index (BMI) were measured through bioelectrical impedance analysis (BIA) using InBody 770. Waist-to-hip ratio (WHR) was measured using measuring tape. Differences between groups and within groups were analyzed using Wilcoxon and Mann-Whitney U test. Spearmans correlation was used to calculate the strength of the correlation between the variables CRP and VFA, BMI and WHR. Partial correlation was used when controlling for gender.

Results

The associations between CRP and VFA, CRP and BMI, CRP and WHR were all considered as medium correlations. Patients with VFA above 137.35 cm^2 showed a significant higher CRP than patients with VFA 137.35 cm^2 or below. Patients with WHR 0.96 or above showed a significant higher CRP than patients with WHR <0.96.

Conclusion

The result of this study suggests that an increasing VFA, BMI and WHR provide a higher CRP level.

Abbreviation list	
OA	Osteoarthritis
VFA	Visceral Fat Area
CRP	C- Reactive Protein
WHR	Waist-to-Hip Ratio
BMI	Body Mass Index
ELISA	Enzyme-Linked ImmunoSorbent Assay
BIA	Bioelectric Impedance Analysis

Abstrakt

Bakgrund

Artros är en vanligt förekommande kronisk sjukdom med komplex etiologi, som påverkas av både genetiska och miljömässiga faktorer. Metabolt triggad inflammation inducerad av fetma, bidrar till aktivering av inflammationsprocessen och högre cirkulerande nivåer av pro-inflammatoriska cytokiner och adipokiner, vilket kan spela en central roll för patofysiologin och utvecklingen av artros. Fetma-relaterade metaboliska faktorer, såsom adipokiner, bidrar till progression av artros genom produktion av pro-inflammatoriska cytokiner samt degraderande enzymer, vilket leder till nedbrytning av ledbrosk och subkrondal benreformering.

Syfte

Syftet med studien var att undersöka sambandet mellan visceralt fett, fetma och en biomarkör för inflammation (C-reaktivt protein, CRP) hos en grupp personer som upplever symtomatisk knäartros.

Metod

Vuxna (n = 89) i varierande åldrar mellan 30-63 år som upplevde symtomatisk knäartros eller var diagnostiserade med knäartros testades en gång. Nivåerna av CRP analyserades med sandwich-ELISA (Enzyme-Linked ImmunoSorbent Assay). Visceral fett area (VFA) och Body Mass Index (BMI) mättes genom bioelektrisk impedansanalys (BIA) genom användning av InBody 770. Midje-höftkvot (WHR) mättes med måttband. Skillnader mellan grupper och inom grupper analyserades med Wilcoxon och Mann-Whitney U-test. Spearmans korrelation användes för att beräkna styrkan i korrelationen mellan variablerna CRP och VFA, BMI och WHR. Partiell korrelation användes vid kontroll för kön.

Resultat

Korrelationerna mellan CRP och VFA, CRP och BMI samt CRP och WHR ansågs vara medium starka korrelationer. Patienter med VFA över 137,35 cm^2 uppvisade ett signifikant högre CRP än patienter med VFA 137,35 cm^2 eller lägre. Patienter med WHR 0.96 eller högre uppvisade ett signifikant högre CRP än patienter med WHR <0.96.

Slutsats

Resultatet av studien tyder på att ökande nivåer av visceralt fett i form av; VFA, BMI och WHR bidrar till en högre koncentration av CRP i blodet.

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Background

Osteoarthritis (OA) is one of the world's ten most disabling diseases in adults aged over 30 years in developed countries (the World Health Organization). Arthritis is the most common form of OA with the knee as the most affected joint (Dell'Isola, et al., 2016). In 2020 Osteoarthritis is estimated to become the fourth leading cause of disability in the adult population (Woolf, Pfleger, 2003). Osteoarthritis is a chronic musculoskeletal disease with high prevalence, and the costs for sick leave due to the disease are significant. In 2000, the cost for knee OA was 1.2% of the total costs for sick leave in Sweden, which corresponds to approximately 460 million Swedish crowns (RFV 2002:2).

Osteoarthritis pathophysiology

Osteoarthritis is characterized by joint pain, impaired mobility, alteration of joint structure including progressive cartilage destruction, synovial inflammation and changes to the subchondral bone (Hosnijeh et al., 2016), which leads to symptoms as chronic pain, joint stiffness, muscle weakness and bone enlargement and swelling (Ashkavand, Malekinejad, Vishwanath, 2013). It is conceived as a complex disease and etiology and new discoveries have differentiated OA into several subgroups/phenotypes; post-traumatic, ageing-related, genetic, symptomatic and metabolic. A specific phenotype in OA can be defined as a collection of observable properties (etiological factors, risk factors) that can identify and characterize a subgroup in a defined population (Dell'Isola et al., 2016). The phenotype metabolic OA has been associated with metabolic syndrome (MetS) and obesity, which can be triggered by metabolic inflammation. Common risk factors for the development of OA include advanced age, injury and mechanical stress, female gender and genetic factors as well as environmental factors (Toussirot et al., 2017).

The etiology of OA has been difficult to understand, but during the recent decades the knowledge about OA has accumulated. The pathogenesis of OA successively changes the tissue homeostasis of articular cartilage and subchrondal bone, and drives the destructive processes (Ashkavand et al., 2013). Chondrocytes are the only cells in cartilage responsible for synthesis and breakdown of matrix and is regulated by

cytokines (cell signaling proteins) and growth factors. During the progression of OA their balance may be disturbed resulting in greater breakdown and loss of cartilage. In OA progression the subchrondal bone becomes stiffer, which leads to lower ability to absorb impact loads which in turn lead to increased stress in the cartilage. Softening of articular cartilage in the patella (chondropathy), causes to erosion of the cartilage. The common features of OA are loss of cartilage, joint space narrowing and hypertrophic bone changes and osteophyte formation (bone spurs/outgrowth of bone) (Ashkavand et al., 2013) (figure 1). In addition to several functional and morphological changes in OA, different studies has shown biochemical factors induced by inflammation, including inflammatory mediators, proteinases, cytokines and proteolytic enzymes, might be important drivers of the molecular mechanism underlying the development of OA (Hosnijeh et al., 2016).

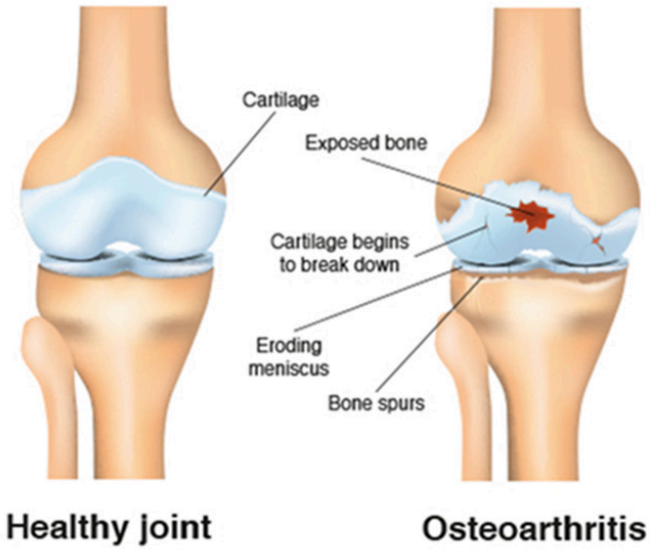

Figure 1. Healthy joint and joint affected by OA (Reumatology Advisor, 2017).

According to Ashkavand et al., (2013) there are numerous different cytokines that in distinctive pathways have impact on articular cartilage metabolism (figure 2). Cytokines are classified in three groups including catabolic (Interleukin-1α, IL-1α, Interleukin-1β, IL-1β, Tumor necrosis factor-α, TNFα), regulatory and enzyme inhibition (Interleukin-6, IL-6), and anabolic (Transforming growth factor-β, TGFβ). IL-1 is a multifunctional pro-inflammatory cytokine that affects most cell types and result in numerous consequences including cartilage breakdown and interference with the activity of growth factors such as IGF (insulin-like growth factor) and decreasing

the synthesis of key matrix components such as aggregan and proliferation of fibroblasts (cell that synthesizes the extracellular matrix and collagen), which have a crucial role in the progression of OA. The occurrence of activated macrophages will release IL, which has an essential role in destruction of cartilage. It is commonly accepted that IL-1 is one of the key cytokines at early and late stages of OA. TNF-α is an effective pro-inflammatory cytokine and has a profound impact in the inflammation process, and matrix degradation by stimulating enzyme secretion from chondrocytes and synovial fibroblasts. The TNF- family also induces the production of both IL-1 and IL-6. Increased levels of these cytokines may contribute to the development of OA. Transforming growth factor beta (TGF-β) is involved in vital biological processes including ECM (Extra Cellular Matrix) synthesis, cell proliferation and tissue repair of articular chondrocytes in the joint. TGF-β released by tissue damage and inflammation triggers cells to form osteophytes, in addition elevated levels of TGF-β have been found in the synovial fluid of OA patients (Ashkavand et al., 2013).

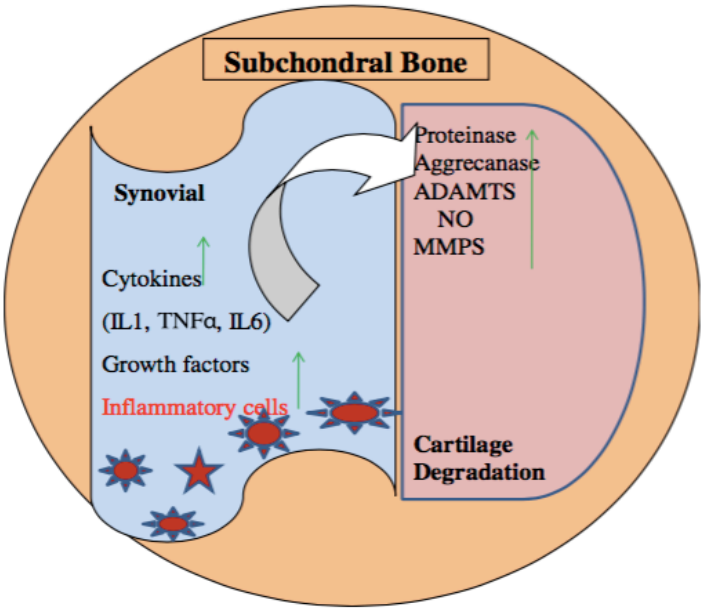

Figure 2. Potential targets for development of OA in knee joint (Ashkavand et al., 2013).

Obesity, adipose tissue and CRP

Inflammation is a necessary response of the immune system to acute infection or trauma, but a prolonged inflammatory state could lead to negative health effects. Abdominal adiposity reflects the amount of visceral fat mass and is more prone to affect the inflammatory process than subcutaneous fat, which is crucial in the development of inflammatory responses and mediators (Wedell-Neergaard et al., 2018). Adipose tissue is metabolically active and is involved in the production of pro-inflammatory adipokines (cytokines produced by fat cells) such as IL-6, TNF- α, IL-1β, IL-8 (Wang el al, 2015). IL-6 and TNF-α are produced by adipocytes and transported by the bloodstream to the liver where they activate the expression of C-reactive protein (CRP) (Srivastava, 2012). C-reactive protein is an acute phase protein and an inflammatory biomarker, which is regulated by pro-inflammatory cytokines. Serum CRP is perhaps the most widely used clinical marker of systemic inflammation (Perruccio et al., 2017). Fat storage, especially in the abdominal regions, is associated with higher circulating levels of CRP and contributes to the development of systemic low-grade inflammation (Panagiotakos et al., 2005). It has been suggested that it may not be the amount of total body fat but the amount of abdominal fat, which is triggering an increase in both chronic systemic inflammation and risk of metabolic disease. Low-grade inflammation is involved in the pathophysiology of a number of aging-related conditions, including OA (Beavers et al., 2015).

To assess body composition and visceral fat area (VFA), Bioelectrical impedance analysis (BIA) can be a suitable tool, because of its simplicity and high validity and reliability. The InBody 770 measures impedance of five segments of the body (trunk, right and left arms, and right and left legs) (Park et al, 2016). InBody 770 has 4 built in electrodes, two at the base where the feet are put and in two handles connected to the device, which are held by the hands. Electrical current with varying frequencies are sent from the electrodes, passing through the body and measuring impedance (electrical resistance) in different body tissues (InBody USA, 2016). Visceral fat can also be measured, quick and precise by using hip-to-waist ratio (WHR), which is a less expensive method. Individuals with high levels of VFA tend to have larger WHR (Pinho et al., 2017). Body Mass Index (BMI) is a common indicator of % body fat; although it is well known that it has an imperfect association depending on the

individuals muscle mass. Muscle mass can vary considerably between individuals of the same height, which contributes substantially to the variability in BMI, especially in leaner individuals (Meeuwsen, Horgan, Meeuwsen, 2010). However, BMI measurement provides an overall image of body fat distribution, while as measurement with VFA and WHR only provide an image of central adiposity.

Osteoarthritis and obesity

Obesity is considered to be a crucial contributing factor in the development of OA. Epidemiological studies have demonstrated a strong association between obesity and OA of weight bearing joints (hip and knee- OA) (Toussirot et al., 2017). These studies also associate obesity to non-weight-bearing joints such as those of the hand. This discovery has led to the idea that besides mechanical factors, systemic and metabolic parameters may contribute to the pathophysiology of OA.

Previous studies have shown that weight reduction reduces the risk of having symptomatic knee OA, because of the decreased strain on the knee joints and reduced amount of total visceral fat (Murphy et al., 2008). There is extensive evidence supporting obesity as one of the main risk factors for knee OA (Reyes et al., 2016). Mechanical overload of the weight-bearing joint and the activation of metabolic factors contributing to joint damage have been proposed as possible mechanisms to explain how obesity increases the risk of OA. Abnormalities in for example body composition, adipokines and different cytokines could lead to the progression and early stages of OA (Wang et al., 2015).

CRP, Osteoarthritis and obesity

Obesity is a strong modifiable risk factor for the development of OA, and is associated with significantly higher levels of CRP (Beavers et al., 2015). Obesity-related metabolic factors, especially adipokines, play an important role in the progression in OA development by inducing pro-inflammatory cytokines and degradative enzymes, leading to structural changes in cartilage and bone remodeling. Studies have proven that high levels of CRP are linked with reduced cartilage volume

and OA progression (Wang et al., 2015).

C-reactive protein is a ring-shaped pentameric protein whose levels rises in response to inflammation, and is found in blood plasma and serum in humans (Black, Kushner, Samols, 2004). Plasma CRP is classified as an acute-phase reactant and biomarker for inflammation because of its rapid and intense increase in concentration, in response to tissue injury or inflammation. CRP is normally present at less than 3 mg/L in blood, in healthy individuals but can rise as much as 1000-fold due to an acute inflammatory stimulus (Macy, Hayes, Tracy, 1997). High- sensitive CRP (hs-CRP) is more precise than standard CRP when measuring baseline concentrations and enables assessment of chronic inflammation. Levels of hs-CRP can be measured by blood sampling and further analysis with ELISA (Enzyme-Linked ImmunoSorbent Assay). The CRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. Followed by an enzyme induced color change in the sample, the intensity of the color is proportional to the concentration of CRP in the collected sample. The absorbance of the color is measured using a spectrophotometer, determining how much light is recorded at 450nm (Heaney, Carrol, Phillips, 2014).

Since knee OA is associated with metabolic abnormalities (such as insulin resistance and impaired glucose tolerance, atherogenic dyslipidemia, hypertension and intra-abdominal adiposity) (Srivastava, 2012), it is relevant to explore biomarkers as CRP together with visceral fat mass area, body mass index and waist-to-hip ratio. Modern approaches for treatment, and also prevention involve early detection and knowledge about early disease and possible associations between OA and other diseases. There are a lack of studies reporting associations between OA and obesity. Because of the increasing incidence of obesity worldwide during the recent decades, associated disorders such as OA have become a serious matter to the current future health. Strategies targeting obesity-related mechanisms like metabolic OA, may be effective in preventing and slowing disease progression of OA.

Aim

The aim of this study was to examine the association between visceral fat, obesity and a biomarker for inflammation (C-reactive protein, CRP) in a group of people experiencing symptomatic knee pain.

Research questions

What is the relation between levels of C-reactive protein and high vs. low visceral fat (area) measured by Bioelectrical Impedance Analysis in subjects experiencing symptomatic knee pain?

What is the relation between levels of C-reactive protein and high vs. low visceral fat (mass) measured by Waist-to-Hip Ratio in subjects experiencing symptomatic knee pain?

What is the relation between levels of C-reactive protein and visceral fat (mass) measured by Body Mass Index in subjects experiencing symptomatic knee pain?

Method

Subjects

Participants were recruited through advertisements in various newspapers (Hallands Nyheter, Hallandsposten, 7dagar and Laholms tidning). In total 89 subjects, 25 men and 64 women of varying ages between 30 and 63 years were included in the study. Inclusion criteria in the study were that the subjects should experience symptomatic knee OA or diagnosed knee OA and be aged between 30 and 63 years. Exclusion criteria were signs of inflammatory rheumatic disease or knee trauma.

Study design

A cross-sectional study to investigate the association between visceral fat and levels of CRP in blood, in patients experiencing symptomatic early knee OA in Halland.

This study is a part of a bigger prospective cohort study by FoU Spenshult in Halland County. The study involves one blood sampling, one measurement of WHR and one measurement with BIA to obtain data about VFA and BMI. All participants gave their written informed consent before the study, as approved by the Research Ethics Committee.

Testing procedure

ELISA

CRP levels above 1 mg/L were analyzed by the department for laboratory medicine at the hospital of Halmstad, using the turbidimetry method with Cobas 8000 (Roche), and results were presented in mg/L. Levels of CRP below 1 mg/L were manually analyzed with the sandwich ELISA method (Enzyme-Linked ImmunoSorbent Assay). High-sensitive CRP (hs-CRP) in serum was measured using hs-CRP ELISA kit with a limit of detection of 0.10mg/L (Abnova, 2017). Serum samples were obtained and stored at -80 °C for the CRP assay. The ELISA assay system uses a unique monoclonal antibody directed against a distinct antigenic determinant on the CRP molecule. This mouse monoclonal anti-CRP antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample with human CRP is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies (figure 3). After a 45-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A reagent, tetramethylbenzidine (TMB) was added and incubated for 20 minutes, resulting in the development of blue color. By adding 100µ HCl, the color development stopped from changing the color to yellow. The concentration of CRP was directly proportional the color intensity of the test sample. Absorbance was measured with a spectrophotometer at 450 nm (Tecan® Sunrise, 2017).

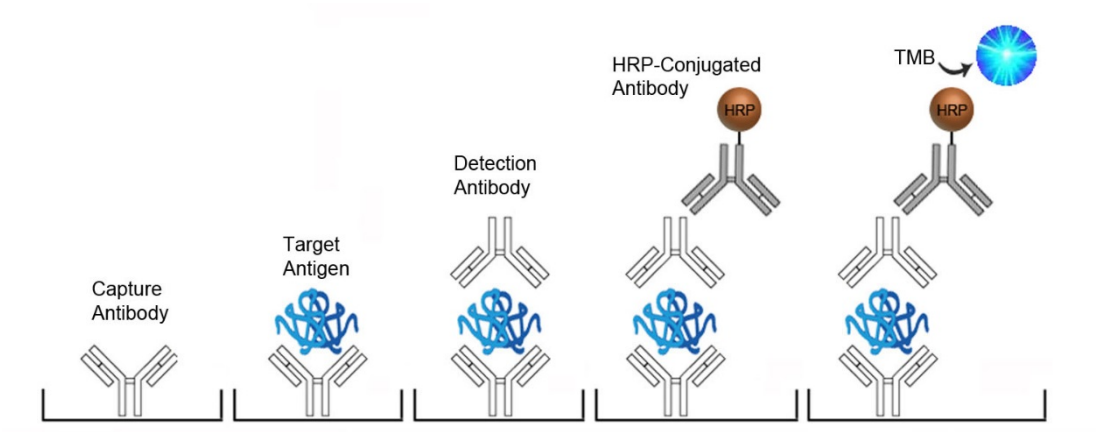

Figure 3. Sandwich ELISA (Enzyme-Linked ImmunoSorbent Assay) (Lifespan Biosciences, 2018).

Bioelectrical impedance, VFA, BMI and WHR

Information about VFA was conducted through BIA with InBody 770. Information about BMI was obtained by using data from Inbody 770. WHR was measured with measuring tape and was determined by dividing the abdomen (cm) with the hip (cm). Standardization of the method, and preparations including information to the subjects, was critical for reliable and valid results. The subjects were therefore informed of the following factors well in advance of the test. Body water tends to gravitate towards the lower body throughout the day, affecting accuracy of the test results. Food and beverages in the stomach and clothes may disturb the measurement of body composition leading to measurement errors. Thus, the measurement should preferably be performed in the morning on an empty stomach, with as little clothes as possible. The subject should stand upright for about 5 minutes before testing. The subjects were also informed to use the bathroom before testing, thus the volume of urine is included in the weight measurement, affecting accuracy of the test results. The subjects were also informed not to exercise one day before the measurement, as this temporarily may change the body composition. Even light exercise can change the subject's body composition temporarily (Inbody USA, 2016). To obtain optimal connection with the body when using InBody 770, the palms and soles were wiped off with the InBody tissues before the test. The subjects were asked to step on the footplate barefoot, whereas weight measurement begun. Personal information about height and age of subject was entered on the device. Thereafter the subjects were asked to hold the handles whereas the measurement could proceed. Time for measurement was about one minute per person. During the Inbody test it was of importance to maintain proper

posture. The subject's arms must not touch the sides of the body, the arms should be kept straight during the test and the thighs should not touch. Measuring body composition with Inbody 770 is considered as a reliable and valid method but to obtain optimal results, the procedure must be performed highly standardized (InBody USA, 2016).

Ethical and social considerations

Oral and written information about the purpose of the study and the test procedure was given to the subjects well in advance of the test. All patients gave their informed consent and the study was performed in accordance with the Declaration of Helsinki. Ethical approval for the present study 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 didn't involve risks, side effects, pain or discomfort towards the subject. Potentially, the measurement of with BIA could have been perceived as integrity impairment. The outcome of the study will hopefully lead to a better understanding of how obesity may affect the development of OA. Deepened knowledge about obesity and its association to OA may be effective in preventing and slowing disease progression of OA. Decreased prevalence of the disease would further reduce the liability on the society, not only socioeconomic, but also physical and psychological.

Statistics

Statistical analyses were performed using SPSS Statistics 21 software. All significant tests were 2-tailed and conducted at the 0.05 significance level because of multiple comparisons. Two splits were made were the patients were divided into 2 groups. First split: group 1 with VFA >137.35 cm^2, and group 2 with VFA 137.35 cm^2 or below. Second split: group 1 with WHR <0.96, and group 2 with WHR 0.96 or above. Two comparisons were made were upper quarter with highest VFA and WHR were compared with the rest of the patients. To test differences between groups the Mann-Whitney U test was used when comparing two groups for continuous variables, because the variables were not normally distributed (Shapiro-Wilks <0.05). For

within-group comparisons, the Wilcoxon test was used. Spearmans correlation was used to calculate the strength of the correlation between the measured variables; VFA, BMI, WHR and CRP levels in blood. Partial correlation was used when controlling for gender. The strength of the correlation was indicated by a standardized correlation coefficient (r) and the coefficient of determination (r^2). Values with r=0 were not be considered as a correlation, r= 0.10-0.29 was considered as a small correlation, r= 0.30-0.49 was be considered as a medium correlation and r= 0.50-1.0 was considered as a strong correlation (Pallant, 2007).

Results

Descriptive characteristics of subjects included in the study

The study included in total 89 subjects, 25 men and 64 women. Median value for age was 61 years (30-63). For all patients median value for CRP was 1.2 mg/L (0.08-10), for VFA 91.2 cm^2 (37-291), for BMI 25.3 (18-48) and for WHR 0.9 (0.1-1.0). The 89 patients were divided into 2 groups, whereas a split was made comparing the upper quarter with highest VFA with the rest of the patients: group 1 (n= 22) with VFA above 137.35 cm^2, and group 2 (n=67) with VFA 137.35 cm^2 or below (table 1). Median value of group 1 was CRP 2.0 mg/L (0.5-10.5). Median value of group 2 was CRP 1.2 mg/L (0.08-10.0). The result shows that group 1 had a significant higher CRP than group 2 (p=0.002) (table 1). Group 1 had a significant higher BMI than group 2 (p=0.001) (table 1). The result also shows that group 1 had a significant WHR than group 2 (p=0.008) (table 1). A second split was made on the upper quarter of WHR, and the 89 patients were divided into 2 groups: group 1 (n=66) with <0.96 WHR, and group 2 (n=22) with 0.96 WHR or above. Median value of group 1 was CRP 1.20 mg/L (0.08-10.50). Median value of group 2 was CRP 1.50 mg/L (0.49-8.10). The result shows that group 2 had a significant higher CRP than group 1 (p=0.030).

Table 1. Body composition and a biomarker for inflammation (CRP) in subjects experiencing symptomatic knee pain.

Variables	**All- median (min-max)**	**Group 1- VFA above 137.35 cm^2**	**Group 2- VFA 137.35 cm^2 or below**	**P-value[a]**
N	89	22	67	
Women % (n)	72 (64)	91 (20)	66 (44)	0.022
Age	61 (30-63)[b]	55 (49-60)	52 (30-63)	0.022
CRP (mg/L)	1.2 (0.08-10)	2.0 (0.5-10.5)	1.2 (0.08-10.0)	0.002
VFA (cm^2)	91.2 (37-291)	180.8 (144-291)	80.1 (37-131)	<0.001
BMI	25.3 (18-48)	30.3 (24-48)	24.2 (18-32)	<0.001
Waist-to-Hip Ratio	0.9 (0.1-1.0)	0.9 (0.8-1.0)	0.9 (0.1-1.1)	0.008
Hip (cm)	102 (79-150)	112.5 (103- 150)	99.5 (79-119)	<0.001
Waist (cm)	94 (10-152)	104 (84- 152)	88 (10- 108)	<0.001

CRP: C-reactive protein; VFA: Visceral fat area, BMI: Body mass index.
[a] P-value, P-values denote the significance of differences between groups. Mann-Whitney U and Wilcoxon test as appropriate
[b] Values are median (min-max) unless other specified.

Correlation between CRP and VFA in subjects experiencing symptomatic knee OA

To assess the association between C- Reactive Protein and the degree of visceral fat area measured with bioelectrical impedance a correlation between the two variables was made. The correlation between CRP and VFA was analyzed with Spearmans correlation on all subjects (n=89). The association between CRP and VFA was considered a medium correlation r= 0.469, r^2= 0.22, p <0.001 (figure 4). Only 22 % of the increase in CRP can be explained by VFA. When controlling for gender the correlation slightly decreased but was still a medium correlation r= 0.411, r^2=0.17, p <0.001.

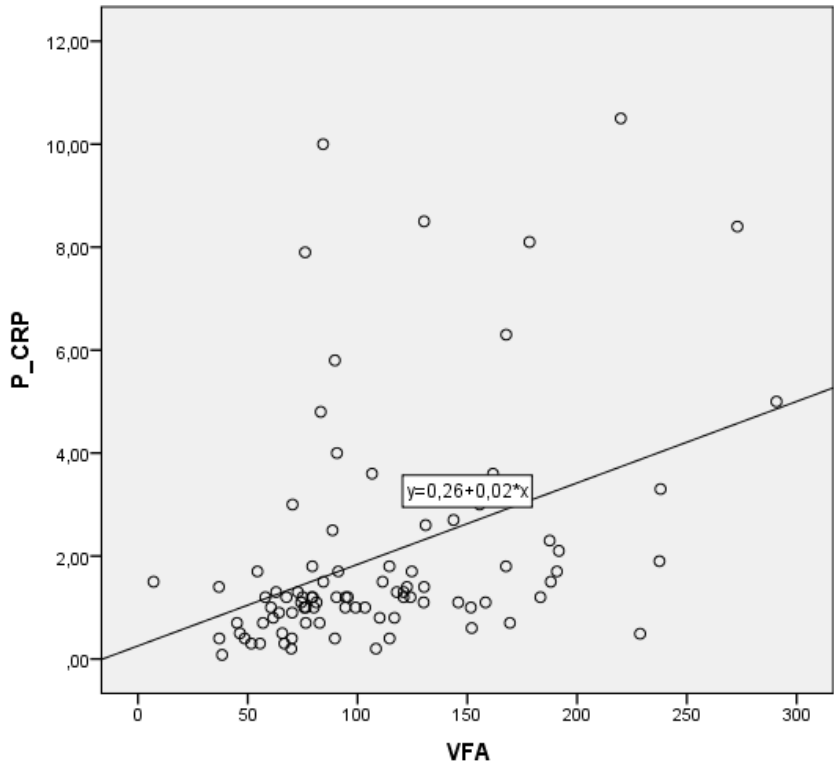

Figure 4. Correlation between P-CRP and VFA, r= 0.469, r^2= 0.22, p <0.001.

Correlation between CRP and BMI in subjects experiencing symptomatic knee OA

To assess the association between C- Reactive Protein and the degree of visceral fat measured with BMI a correlation between the variables was made. The correlation between CRP and BMI was analyzed with Spearmans correlation on all subjects (n=89). The association between CRP and BMI is considered to be a medium correlation r= 0.446, r^2= 0.20, p<0.001 (figure 5). Only 20 % of the increase in CRP can be explained by BMI. When controlling for gender the correlation slightly decreased but was still a medium correlation r= 0.390, r^2= 0.15, p<0.001.

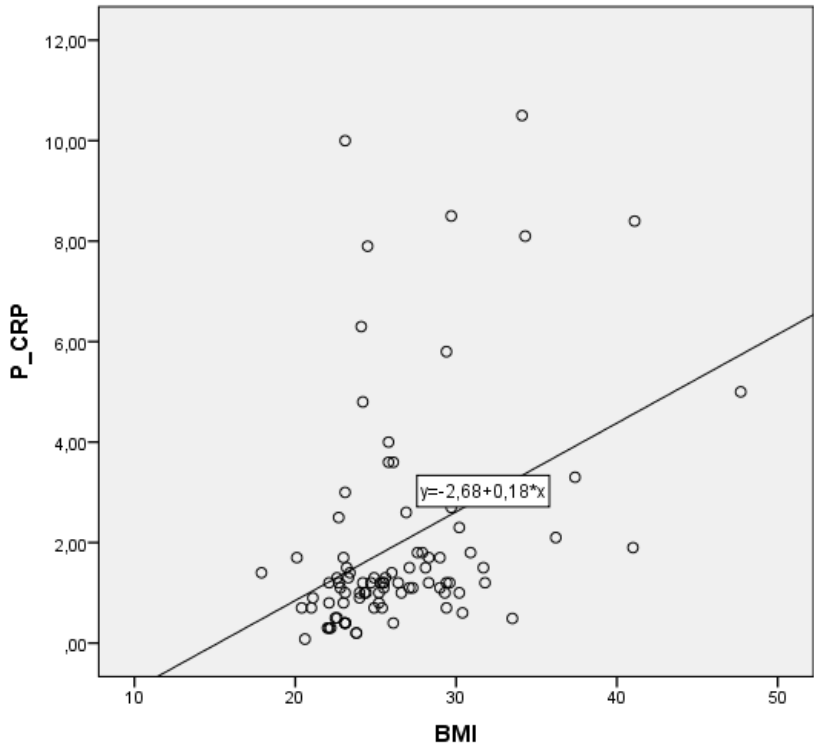

Figure 5. Correlation between P-CRP and BMI, r= 0.446, r^2= 0.20, p<0.001.

Correlation between CRP and WHR in subjects experiencing symptomatic knee OA

To assess the association between C- Reactive Protein and the degree of visceral fat measured with waist-to-hip ratio a correlation between the variables was made. The correlation between CRP and WHR was analyzed with Spearmans correlation on all subjects (n=89). The association between CRP and WHR was considered a medium correlation r= 0.307, r^2= 0.09, p=0.004. Only 9 % of the increase in CRP can be explained by WHR. When controlling for gender the correlation decreased and was considered a small correlation r= 0.166, r^2=0.03, p=0.124 (figure 6).

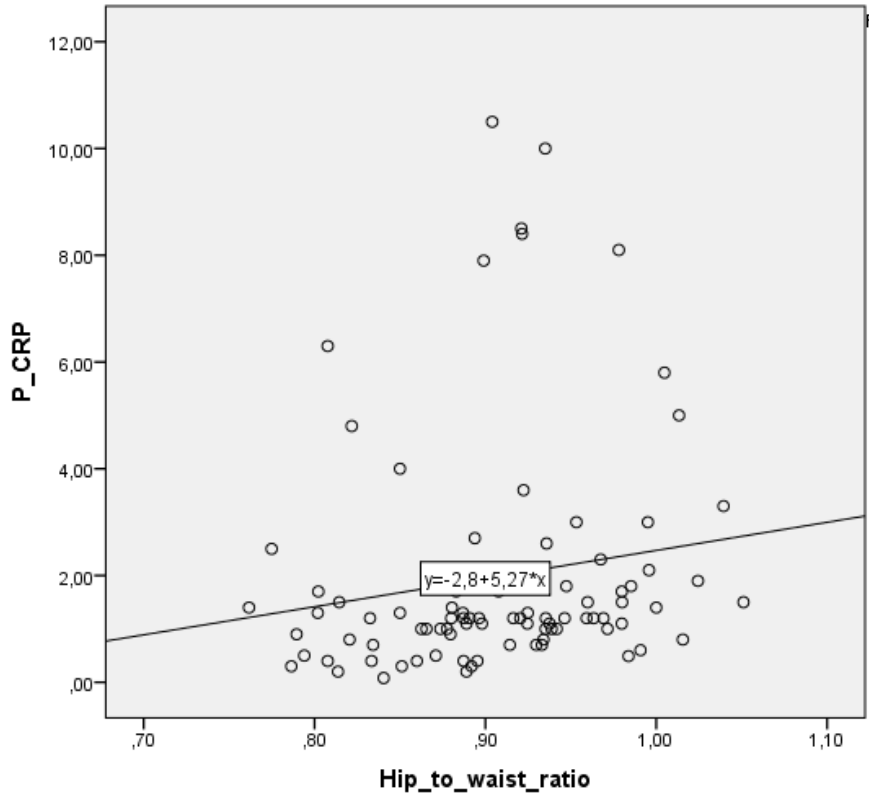

Figure 6. Correlation between P- CRP and WHR, r= 0.307, r^2= 0.09, p=0.004.

Discussion

Result

The purpose of this study was to examine the association between visceral fat, obesity and a biomarker for inflammation (C-reactive protein, CRP) in a group of people experiencing symptomatic knee pain. The results suggest that an increasing VFA, BMI and WHR provide a higher CRP level in these patients. Although, only 22 %, 20 % respectively 9 % can be explained by VFA, BMI and WHR. When controlling for gender, all correlations between CRP and VFA, BMI and WHR slightly decreased.

Similar findings were described by Spector et al. (1997) who also found a significant increased inflammatory response trough enlarged CRP levels, in patients with early knee OA, compared to patients with no knee OA. Spector et al. (1997) also suggested higher CRP-levels predict those whose disease will progress over 4 years, proposing that low-grade inflammation may be a significant aspect of early OA and may be

amenable to therapeutic intervention and secondary prevention. Pearle et al. (2007) found that plasma levels of hs-CRP in hip or knee OA patients with inflammatory infiltrates in synovial membrane were significantly higher than those without inflammation. Similar significant correlations between hs-CRP and BMI as presented in the current study, were also found by Pearle et al, (2007). Wedell-Neergaard et al. (2018) described a significant positive association between central adiposity through waist circumference and hs-CRP, in both men and women, independently of BMI. Other studies have shown that individuals can be obese and metabolically healthy (high insulin sensitivity, low abdominal adiposity and low inflammation level) or of normal weight with an unhealthy metabolic profile (Wedell-Neergaard et al., 2018).

Women can have a heightened inflammatory response compared with men (Straub, 2007). According to Perruccio et al. (2017) the prevalence of OA also tends to be greater amongst women than men. As well, women often report greater musculoskeletal pain, including OA pain, than men. Perruccio et al. (2017) suggest an important connection between pain and inflammation, and found a higher median CRP concentration in women than men (15.4 vs. 9.3 mg/l). In the present study, the majority of the patients were women (72%), which potentially could have affected the outcome of the results. Smoking induces inflammatory responses and contributes to low-grade inflammatory mechanisms, which promote elevated levels of inflammatory biomarkers such as CRP. Ohsawa et al. (2005) found that levels of CRP in current smokers were elevated, but unrelated to the number of cigarettes smoked per day. In conclusion, it was mentioned that past smokers and long-term smoking cessation might contribute to the reduction in risk of development of cardiovascular diseases through inflammatory mechanisms. The common cold is another factor that contributes to increased levels of CRP in blood (Whicher et al., 1985). Whicher et al. (1985) found a highly significant increase of CRP concentrations in blood. Control for the common cold or any other sickness and smoking did not occur in the present study, which potentially could have led to elevated levels of hs-CRP in possibly smoking, or recently sick patients and misleading results.

Recent studies highlight the role of adipose tissue in the development of a systemic inflammatory state that contributes to obesity-associated cardiovascular risk (Berg,

Scherer, 2005). Circulating mediators of inflammation (e.g. CRP) participate in the mechanisms of developing cardiovascular disease, and many of these inflammatory proteins are secreted directly from adipocytes and adipose tissue. Berg et al. (2005) suggest that secretion of inflammatory-induced factors from visceral adipose tissue may contribute to the increased cardiovascular risk associated with obesity. Other studies support this evidence where having OA and elevated levels of CRP is associated as a risk factor for development of further diseases. According to Nishide et al. (2015) excessive levels of visceral fat induces disorders such as cardiovascular disease and diabetes. Hs-CRP is well known as a marker of low-grade inflammation and has attracted recent attention as a sensitive marker for cardiovascular diseases. Elevated hs-CRP is associated with obesity and abnormal lipid metabolism in adults. Further, hs-CRP concentrations were found to inversely correlate with the concentrations of adiponectin, a protein that enhances insulin sensitivity and prevents atherosclerosis (Nishide et al., 2015). An association between CRP elevation and future major cardiovascular events has been recognized, leading to the recommendations by the Centers for Disease Control and the American Heart Association, which indicates that patients at intermediate risk or coronary heart disease might benefit from regular measurement of CRP (Kushner et al., 2004). Evidence for a connection between obesity and inflammation has also been found in the context of clinical weight loss studies. Whether the weight loss is attributable to decreased dietary intake, increasing fuel use through exercise or other methods, loss of adipose tissue is associated with a decrease in pro-inflammatory biomarkers such as IL-6, CRP and TNF-α (Berg et al., 2005).

Method

When measuring VFA and BMI with Inbody 770 the patients wore light clothes, which could be a possible limitation in the study. According to Hurst et al. (2014) wearing tight clothes (close to the body) is the most optimal to obtain valid and reliable result. The importance of standardized performance in the method is also mentioned, where the preparations and information to the subject is of great importance for valid and reliable results (Hurst et al., 2014). Hurst et al. (2014) tested the reliability and validated the BIA method against Air displacement

plethysmography (ADP) and dual-energy X-ray absorptiometry (DXA), the gold standard for measuring body composition. Results showed excellent relative agreement to the estimated true value ($\rho = 0.97$ (0.96, 0.98) in measuring body fat.

Another possible limitation was that measurement of WHR was performed by several different individuals, which could have affected the results due to slightly various implementations of the method, and exact positioning of the measurement on the patients body. Further, this could question the inter-rater reliability of the present study.

The execution of ELISA and its pipetting is considered a critical moment, as the method involves a number of parts that require specific knowledge about the method, experience and proper performance, such as precision of using a pipette. Space is given for errors in pipetting due to the human factor, therefore correct pipetting is considered to be essential to obtain reliable and valid results. Other possible internal weaknesses using an ELISA kit could be the kit manufacturer and the quality of the antibodies.

Conclusion

The result of this study suggests that an increasing VFA, BMI and WHR provide a higher CRP level, and strong correlations between central adiposity and inflammation process (levels of CRP). Thus, there are numerous interacting causalities that were not controlled for in the study, but also could affect/explain the increasing CRP level. The increasing occurrence of obesity worldwide during the recent decades, have led to a rise of associated disorders such as OA, and have become a serious matter to the future health. Methods targeting obesity-related mechanisms like metabolic OA, may be efficient in preventing and slowing disease progression of OA. Strategies for treatment and prevention involve early detection and knowledge about disease and possible associations between OA and other diseases. Although, this study suggest a positive relationship between inflammatory biomarkers (CRP) and obesity, further research is necessary to broaden and deepen the knowledge about the relationship

between obesity, low-grade inflammation and development of OA.

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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, 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 undersökningstillfällena kommer följande att utföras:

- Dina knän undersöks och du får göra ett test där man mäter styrkan i ben och händer. Man kommer även att göra en smärtundersökning där man trycker på förutbestämda punkter på kroppen för att få en bild av om du har smärta någon annanstans på kroppen. Blodtryck, vikt, längd, bukomfång och kroppssammansättning dvs andel muskler/fettväv kommer också att mätas.
- 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 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

Dina svar och dina resultat kommer att behandlas så att inte obehöriga kan ta del av dem. Sammanställningen av enkätsvaren sker i avidentifierad form, vilket betyder att ditt namn och personnummer ersätts av en kod. Alla resultat kommer att redovisas i grupp, dvs inga enskilda patientresultat kan identifieras. FoU Spenshult är forskningshuvudman och ytterst ansvarig för behandlingen av personuppgifter, vilket skyddar din identitet i enlighet med sekretesslagen och sekretessförordningen. Undersökningen är granskad och godkänd av etikprövningsnämden i Lund. Biobanken finns på FoU Spenshult, all provhantering görs av biobanksansvarig. Alla prover förvaras kodade, kodnyckeln förvaras inlåst, skilt från blodproverna. Proverna kan inte utan tillgång till en kodnyckel direkt hänföras till en individ. Proverna kommer endast att användas på det sätt som beskrivits och du har samtyckt till. Om proverna i framtiden skulle kunna användas i ännu ej planerad forskning kommer en ny etisk prövning att göras samt efter beslut i etikprövnings-nämnden kan du komma att kontaktas igen.

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:

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Bitr. forskningschef vid FoU Spenshult

Samtyckesblankett

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

☐ 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

E-post:_____

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