

TECHNISCHE UNIVERSITÄT MÜNCHEN

Klinik für Orthopädie und Unfallchirurgie
des Klinikums rechts der Isar
(Direktor: Univ.-Prof. Dr. R. Gradinger)

Adipokine Production in the Infrapatellar Fat Pad in Osteoarthritis

Ulrike A. Dapunt

Vollständiger Abdruck der von der Fakultät für Medizin der Technischen Universität München zur Erlangung des akademischen Grades eines
Doktors der Medizin
genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. D. Neumeier

Prüfer der Dissertation:

1. Priv.-Doz. Dr. R. H. H. Burgkart
2. Univ.-Prof. Dr. A. Imhoff

Die Dissertation wurde am 29.04.2010 bei der Technischen Universität München eingereicht und durch die Fakultät für Medizin am 22.09.2010 angenommen.

Index

Index.....	1
1. Working hypothesis.....	3
2. Introduction.....	5
2.1 The Infrapatellar Fat Pad.....	5
2.1.1 Anatomy.....	5
2.1.2 Hoffa Disease.....	6
2.2 Osteoarthritis.....	7
2.2.1 Definition.....	7
2.2.2 Etiology.....	8
2.2.3 Prevalence.....	8
2.2.4 Epidemiology.....	9
2.2.5 Pathogenesis.....	14
2.3 Obesity- a state of chronic low-grade inflammation.....	16
2.4 Adipokines.....	20
2.4.1 Leptin.....	20
2.4.2 Adiponectin.....	22
2.4.3 IL-6.....	24
2.4.4 IL-8.....	26
2.4.5 TNF- α	27
2.4.6 MCP-1.....	28
3. Patients and Methods.....	30
3.1 Patients.....	30
3.2 Isolation and culture of adipocytes.....	30
3.3 Measurement of adipokine release.....	31
3.4 Statistical analysis.....	32
4. Results.....	33
4.1 Weight of the infrapatellar fat pad.....	33
4.2 Adipocyte volume of adipose tissue samples.....	33
4.3 Adipokine-secretion of infrapatellar fat pad, control fat and synovial membrane.....	34
4.3.1 IL-6.....	34
4.3.2 IL-8.....	36
4.3.3 TNF- α	38
4.3.4 MCP-1.....	42

4.3.5 Adiponectin.....	44
4.3.6 Leptin.....	46
4.4 Adipokines in synovial fluid.....	51
5. Discussion.....	53
5.1 Osteoarthritis and cytokines.....	53
5.1.1 Adipose tissue and inflammation.....	53
5.2 Fat cell size of Hoffa and control fat and their correlations with clinical parameters..	55
5.3 Evidence of adipokine production in the infrapatellar fat pad.....	56
5.4 Adipokines in synovial fluid in correlation with the release from the Hoffa fat pad...	57
5.5 Production of cytokines from other structures in/surrounding the knee.....	59
5.6 Cytokine production in the synovial membrane.....	61
5.7 Osteoarthritis- a systemic disease?.....	62
6. Summary.....	64
7. Literature.....	66
8. List of tables.....	75
9. List of abbreviations.....	77
10. Acknowledgments.....	78

Adipokine Production in the Infrapatellar Fat Pad in Osteoarthritis

1. Working hypothesis

Recently more and more attention has been drawn to the fact that fat tissue is a secretory active organ. We now know for sure that an increased amount of body fat leads to a chronic low-grade inflammation in the body due to the (pro-)inflammatory parameters secreted in fat tissue.

It was therefore of great interest to us to investigate the function of the infrapatellar fat pad further. As osteoarthritis is also considered to be an inflammatory disease it seemed plausible, that the infrapatellar fat pad might secrete inflammatory agents into the knee joint and thus support the advance of the latter disease.

Therefore, the aim of this study was to investigate the influence of so-called adipokines from the infrapatellar fat pad on the pathogenesis of osteoarthritis.

We were interested in the following questions:

1) Is there evidence of adipokine-production in the infrapatellar fat pad and does the production relate to concentrations present in the synovial fluid?

First of all we wanted to find out whether the infrapatellar fat pad is at all a secretory active organ. But furthermore, a positive correlation between the amount of adipokines found in synovial fluid and in the fat pad could indicate an active release of adipokines into the joint space.

2) Are adipocytes from the fat pad different than adipocytes from white adipose tissue of the thigh (size, secretory activity)?

We know that there are several different types of fat. As very little research had been done on the infrapatellar fat tissue, we were interested to compare these adipocytes to those of fat from the thigh.

3) Does the fat cell size and secretory activity correlate with clinical parameters (BMI, etc.)? Some fat cells increase in size and their secretory activity simultaneously with the BMI. We wanted to find out which mechanisms influence the infrapatellar fat pad.

4) Do other structures surrounding the knee (white adipose tissue of the thigh and synovial membrane) also produce adipokines?

In order to get a better understanding of how much adipokines are actually produced in the infrapatellar fat pad, we compared them to the productions found in white adipose tissue from the thigh and in the synovial membrane.

5) Can we conclude that cytokine production in the infrapatellar fat pad stimulates the cytokine production in the synovial membrane?

Another possibility is that adipokines from the infrapatellar fat pad not only damage structures of the knee, but also stimulate further release of inflammatory parameters in the synovial membrane.

6) Can we conclude from the results that the damaging effects of osteoarthritis are further supported by proinflammatory adipokines produced in the infrapatellar fat pad?

2. Introduction

2.1 The Infrapatellar Fat Pad

2.1.1 Anatomy

The infrapatellar fat pad (IFP) is often referred to as Hoffa's pad according to Albert Hoffa, who first described it in a paper in 1904 (53). The IFP is one of three fat pads found in the anterior knee joint compartment, the others being the quadriceps and pre-femoral fat bodies. It is an intra-capsular but extrasynovial structure, covered by synovial membrane dorsally. Anteriorly of the IFP lies the patellar tendon, posteriorly the femoral condyles and intercondylar notch, superiorly the inferior pole of the patella and inferiorly the anterior horns of the menisci (Images 1 and 2).

The IFP has attachments to the inferior pole of the patella, the proximal end of the patellar tendon and to the anterior horns of the medial and lateral menisci. Furthermore, the ligamentum mucosum is attached to the fat pad inferiorly. The ligamentum mucosum consists of two folds of synovial membrane covering the IFP and converges as a single band that continues to the front of the intercondylar fossa of the femur. The free edges of the ligamentum mucosum are called alar ligaments and include the lateral margins of the IFP. The size and volume of the IFP vary considerably. According to Gallagher et al. (44), the mean volume of the fat pad is 24 ml (range 12–36) and the mean difference between subjects' right and left knees is 4 ml (range 2–7). It usually consists of two large basal prominences lying on either side of the intercondylar notch, with three to six alar projections emanating from each basal prominence.

The blood supply of the IFP is derived from the transverse infrapatellar anastomosis of the medial and lateral inferior genicular arteries. The vascularity of the IFP is abundant and stretches out into a weblike network of vessels that permeate the entire fat pad. Venous drainage is accomplished by the genicular venous plexus.

Innervation of the IFP is provided by the popliteal plexus, which is formed by the posterior articular nerve. The latter is a branch of the posterior tibial nerve and arises above the knee in the popliteal fossa.

Histologically, the IFP is composed of a stroma of connective tissue that supports the uniformly arranged fat cell (30,69,110,44). Next to adipocytes, adipose tissue contains several other cell types which include fibroblasts, macrophages, and endothelial cells. Adipose tissue also contains many small blood vessels.

The function of the IFP has long been debated. MacConaill (67) suggested that fat pads occupied dead space in a joint, maintaining the joint cavity and promoting efficient lubrication. On the other hand, Presle et al. (87) were able to show, that the IFP is a source of cytokine production and thus making it questionable whether the IFP merely serves frictionless movement in the knee joint.

2.1.2 Hoffa Disease

The IFP has been discussed extensively in the context of anterior knee pain (69). Among the causes of this disease, the entrapment of portions of the IFP between the femoropatellar and femorotibial articular surfaces is thought to play an important role. As this disease was first described by Hoffa, it is also termed “Hoffa disease”. Patients with anterior knee pain typically complain about intermittent burning peripatellar pain which is often triggered by dynamic extension (stair climbing) or prolonged flexion of the knee (cinema, car driving). Sometimes a swelling of the soft tissue on both sides of the patellar tendon occurs. 85% of the cases are caused by contusive trauma. Two phases can be distinguished: the acute and the chronic phase. Recently, a study by Bohnsack et al. (10) found a high number of S-100- and substance-P nerves in the synovial membrane of the anterior knee compartment and in the IFP, suggesting that anterior knee pain could indeed originate in compression or chronic inflammation of those tissues. According to them, a substance-P-mediated hyperperfusion by vasodilatation, an extravasation of plasma proteins and an adhesion of leukocytes could cause edema of the fat pad. Chronic edema of the IFP is considered the key pathology of the Hoffa disease, leading to soft tissue entrapment ischemia and in consequence to a lipomateous tissue necrosis in the long term.

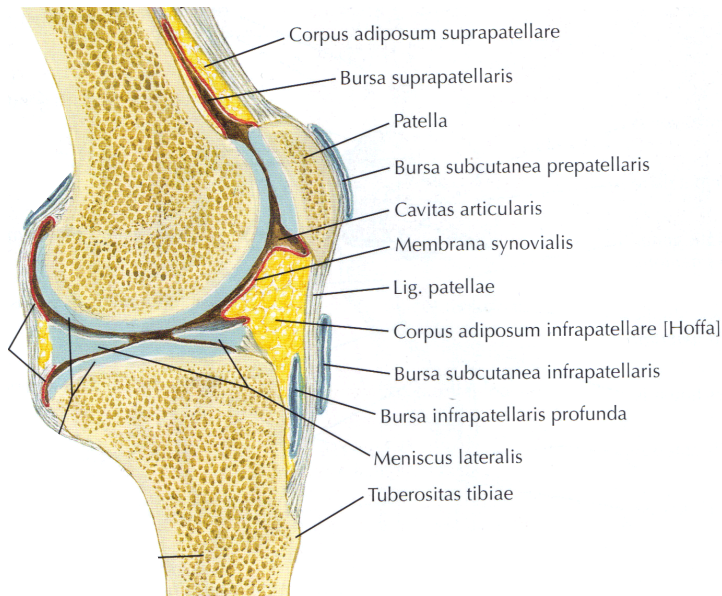


Image 1: Sagittal demonstration of the knee joint showing the location of the IFP.
 Elsevier 2006. F.H. Netter: Atlas of Human Anatomy.

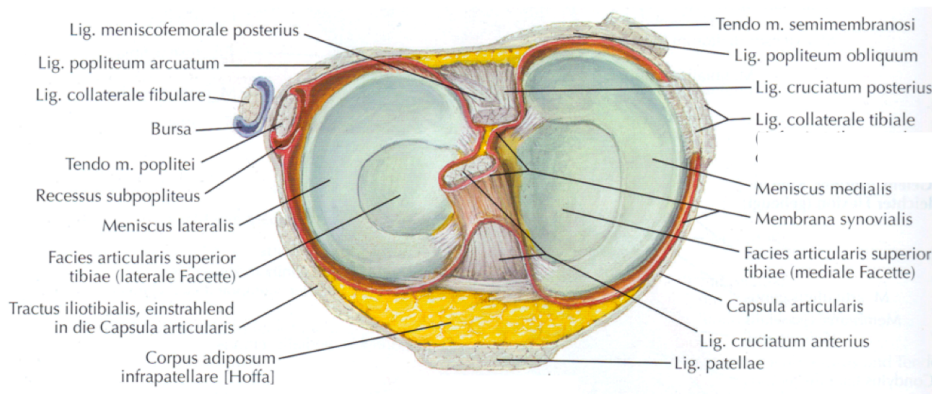


Image 2: Axial demonstration of the knee joint showing the location of the IFP.
 Elsevier 2006. F.H. Netter: Atlas of Human Anatomy.

2.2 Osteoarthritis

2.2.1 Definition

Osteoarthritis (OA) also named degenerative joint disease or osteoarthrosis (because of the inconsistency of inflammation). It is a disease of the whole knee joint in which all articular structures are affected. The pathology of OA affects the joint in a degenerative process that includes focal and progressive articular cartilage loss with concomitant changes in the underlying bone and the development of osteophytes. Among the various different types of

arthritis conditions (i.e. rheumatoid arthritis, psoriatic arthritis, etc.), OA is the most common type (87,36).

2.2.2 Etiology

In OA, a primary and a secondary type can be distinguished. Primary osteoarthritis is commonly thought to be caused by a degradation of the cartilage tissue resulting in decreased resistance for stress imposed on a joint. Exact mechanisms are still unknown. Secondary OA on the other hand is caused by other, well-known factors or diseases (i.e. hip dysplasia, Perthes disease, hyperparathyroidism, post-traumatic etc.) but the resulting pathology imposes clinically the same as for primary OA.

2.2.3 Prevalence

OA is an extremely common disease of the elderly. As life-expectancy is increasing, also the incidence and prevalence of this illness are rising. It has to be considered that many elderly do not have OA, suggesting that it is not a universal feature of aging.

According to the German federal office for statistics, there are currently about 5 million patients in Germany who suffer from symptoms of OA in the knee. The prevalence of radiographically defined OA has been estimated in a study representing the US population: the NHANES I (National Health and Nutrition Survey) study showed that there was radiographic evidence of OA in more than 70% of persons aged 55 to 74 years (typical radiographic evaluation considers narrowing of the joint space, subchondral sclerosis, and osteophyte formation) (63). Nevertheless, individuals with radiographic evidence of OA may be asymptomatic at any time. In another American subpopulation, only 65% of those older than 55 years with radiographic evidence of knee OA reported knee pain (18).

Another feature of osteoarthritis is the higher prevalence in women. They also show a more generalized distribution in all joints than men. Before the age of 50, men have a higher prevalence of the disease than women, possibly owing to athletic and other joint injuries in young men. After the age of 50, women show a much higher prevalence of joint diseases than men. The reasons for this gender difference are unclear but may be related to the hormone deficiency that develops in women around the age of 50 years.

OA affects certain joints while sparing others. For example, in the hands, the distal interphalangeal, proximal interphalangeal, and carpometacarpal joints are frequently affected, whereas the wrists only rarely show signs of inflammation. Other frequently affected joints include the cervical and lumbosacral spine, as well as the hip and knee joint. The ankles and elbows are usually spared (36). Two theories have been proposed to explain why certain joints are spared from OA while others are more frequently affected.

The first theory is that the joints were not designed for walking erectly. With evolution to bipeds, shifting of weight was limited to the lower extremities, and pincer grip capability developed. As a result, OA occurs in joints that are maldesigned for man's current habitus, including lower extremity joints involved in weight bearing and in holding man upright, such as the spine, hip and knees, and joints involved in pincer grip in the hand (55). Another explanation may be that some joints are more resistant to stress that might induce OA than others (31). This explains why the ankle joint, even though it is weight bearing and subject to considerable stress, is less likely to develop OA than the knee joint. Evidence supporting this latter hypothesis includes the finding that cytokine-mediated injury does less likely damage the ankle joint, whereas the knee joint cartilage is commonly affected (36).

2.2.4. Epidemiology

The joints are permanently under moving stress. Whether this use constitutes a dynamic trophic and healthy effect on joints or an injurious effect depends on the activities involved and the vulnerability of the underlying joint. Most daily activities, even if performed repeatedly over many years, do not produce sufficient injury to a joint to cause OA, at least not if the joint is healthy. Joint vulnerability is a key concept in understanding when a joint is susceptible and when it is not. Joints become susceptible to injury and subsequent OA when local factors act within the context of systemic susceptibility (93,36).

Local Factors

Local factors determine the risk of OA and make joints vulnerable to normal daily activity (93,36). These factors may be further characterized as extrinsic or intrinsic to the joint organ. Factors and events are designated as extrinsic if they have an origin outside the joint itself and

its immediate environment - for example, physical activity and injury. More recently, intrinsic factors such as strength, laxity, and proprioception have begun to receive more attention.

Extrinsic Factors

-Physical activity

In a normal young joint in which vulnerability is usually minimal, normal daily activities would unlikely cause any injury. But a highly stressful activity may overwhelm the ability of a normal joint to deal with stress. In an older, more vulnerable joint, activities that may be well tolerated in normal joints may cause joint injury. Evidence suggests that hard physical activity in older female subjects causes a high rate of OA (75), whereas the same level of activity has little effect on OA development in middle-aged women (36).

-Injury

A severe injury to a joint, such as an athletic injury, or an injury in which there is a fracture of a bone near a joint can cause permanent damage to many of the structures within a joint and can serve as the incipient lesion of OA. A severe damage would alter the biomechanics of the joint, increasing the stress across a local area and would thereby dramatically increase the disease risk. Examples of such injuries include meniscal tears and cruciate ligament tears (20,19).

For some joints, such as the ankles, which are rarely affected by OA, most diseases are ascribable to such a major joint injury. An important interplay occurs between systemic and local joint vulnerabilities. Injury to the knee is, if at all, unlikely to cause OA in an adolescent rapidly. In contrast, injury to the knee after the age of 30 produces rapidly progressive OA, suggesting that the older joint is more vulnerable to major injury than the younger joint (36).

Intrinsic factors

-Alignment

Knee alignment influences the natural risk of subsequent knee OA progression. At the knee, alignment (the hip/knee/ankle angle) is a key determinant of stress distribution. Varus alignment was associated with an increase in the odds of medial OA progression over 18

months. Valgus alignment at baseline was associated with a comparable increase in the odds of lateral OA progression (36,93).

-Muscle strength

Available studies support the hypothesis that muscle forces have complex effects in OA. Coordinated muscle activity attenuates load. However, certain muscle forces may be associated with increased joint reaction force.

Because muscular contraction is a major source of joint force, it stands to reason that excess contraction, especially in a joint that might be misshapen or mal-aligned, could actually injure the joints. Whether this might actually be the primary cause for OA is still very unclear. On the other hand, it seems plausible that muscle strength might be altered as a consequence of OA. Patients with OA have weakness in the muscles that bridge the diseased joint. At first, affected persons become relatively inactive, leading to muscular atrophy. Secondly, direct neurologic messages transmitted by a swollen joint through afferent impulses inhibit the maximal contraction of muscles bridging joints, leading to apparent weakness. This weakness most likely accounts for much of the disability and may increase the risk of joint destruction. Muscle strengthening and exercise are effective treatment modalities, lessening patient disability (36,93).

-Varus-valgus laxity

In osteoarthritic knees, a varus-valgus laxity has been found to be associated with the disease. It has been speculated that the laxity is not exclusively the consequence of late-stage OA pathology and that some portion of the laxity predates the disease. However, changes over the course of OA are very likely to further affect varus-valgus stability.

It is likely that osteophytes prevent even greater instability. At earlier stages of OA, osteophytes effectively contribute to varus-valgus stability. Although osteophytes still have some stabilizing activity at advanced states, they cannot prevent further increases in varus-valgus laxity.

Systemic factors

If one knee, hip, or hand is affected by OA, there is a greater likelihood of contralateral OA (37,93). In the National Health and Nutrition Examination Survey I cohort bilateral knee OA was more than twice as common as an unilateral form. In other studies 34% of women with

unilateral knee OA developed contralateral OA within 2 years (99), and 92% with unilateral OA developed contralateral OA over 11 years (98). The observation of such patterns of OA led investigators to conclude that systemic factors must be central to OA development.

However, it needs to be mentioned, that local factors may also occur bilaterally. For example, there is evidence that varus-valgus laxity, alignment, proprioceptive accuracy, and strength are very similar in right and left knees.

Unmodifiable factors

Well established systemic risk factors for OA include aging, obesity, gender, and, in selected subgroups, congenital anomalies. Less well established risk factors include bone density, nutrients, particularly those that function as antioxidants, and genetic factors. Only some of these will be discussed here in more detail (96).

Nutrients

Because oxygen radicals have been identified as potent agents in the destruction of cartilage and connective tissue (97,51), attention has been directed toward the use of the antioxidant vitamins A, C, and E as means of preventing or ameliorating the pain and disability of OA. The production of free radicals can be the consequence of tissue damage associated with arthritis, leading investigators to believe that oxidative stress contributes to the progression of arthritis rather than the initiation of the disease. An additional nutrient, vitamin D, may also play a potential role in the course of OA via bone mineralization and cell differentiation, paralleling the actions of vitamin A and vitamin C (74).

It is apparent, that vitamin D deficiency is a common disorder, especially in the elderly (74). The Framingham investigators have reported that a relative deficit of vitamin D, as determined by dietary intake and serum levels, is present and predisposes patients to OA of the knee. They reported that persons with low dietary vitamin D intake and lower serum vitamin D levels were approximately three times more likely to exhibit progression of established knee OA than those having higher intakes of dietary vitamin D and higher levels of serum vitamin D. However, they found no evidence that lower dietary intakes and lower serum levels influenced the risk for development of OA in a previously normal knee (74,36,96).

Hormone-related risk factors

There is an ongoing debate as to whether gonadal hormones contribute to the pathogenesis of OA. This debate has focused largely on the role of levels of estradiol, the major estrogen in premenopausal women. Concerning this, some investigators suggested a protective effect for higher levels and other investigators identified a negative role (90,81). Women have a high risk for OA after the age of 50 years, much higher than the risk for men (relative risk 9.2% vs 3.9%) Studies have been inconsistent regarding to whether estrogen replacement therapy eliminates that risk or not. Potential mechanisms for estrogen in the pathogenesis of OA include an indirect role in maintaining bone density, a direct role of estrogens and estrogen receptors in collagen synthesis including its suppression, and an indirect role for estrogen in modulating the excess expression of cytokines (96,36,90,81).

Obesity

Obesity is a well-recognised risk factor for OA of the knee (72, 52, 103, 43, 48). Persons who are overweight have a higher prevalence of knee OA than persons who are not overweight, and being overweight precedes the development of knee OA. The association between obesity and OA of the knee is stronger for bilateral than unilateral disease and more marked in women than in men. Prospective data on women suggest that the risk for knee OA is increased by approximately 15% for each additional unit of BMI above 27 kg/m² (4). There is some evidence that weight loss reduces the risk of subsequent symptomatic knee disease (52). Furthermore, a study by Toda et al. (106) showed, that a loss of body fat mass was more closely related to a relief of symptoms than simply a loss of body weight.

Body weight could act through several mechanisms to contribute to the development of OA. Naturally, being overweight increases the amount of force across a weight-bearing joint. In addition, adipose tissue may produce atypical amounts of hormones or growth factors that potentially affect cartilage integrity or the underlying bone, predisposing them to OA development (96,36,87). This theory might be supported by the fact that the risk factor for developing OA in non-weight-bearing joints, such as hands, was also shown to be associated with BMI (80).

In conclusion, it can be hypothesized that local and systemic factors most likely act together in mediating osteoarthritic changes. For example, a varus malalignment will intensify the effect of excess body weight on the medial compartment of the knee (93).

2.2.5 Pathogenesis

Early in the disease, the pathologic events are dynamic. Injured cartilage mounts an attempt to increase matrix synthesis and repair while exuberant osteophytes stabilize the joint. In a later stage there are irreversible pathologic changes in most of the joint structures. Therefore, OA is best conceptualized as a total joint failure. The transition from a dynamic to an irreversible process varies greatly from joint to joint and person to person. These notions of disease describing it as dynamic and affecting all joint structures replace the concept of OA as being inevitably progressive and affecting hyaline articular cartilage predominantly. Hyaline articular cartilage loss is a signature event in OA. In synovial joints, articular cartilage provides protection for the subchondral bone against mechanical loading and shearing forces. This highly specialized connective tissue consists principally of water, collagen, and proteoglycan (PG). Its conformation creates a stiff fiber-reinforced water gel with resilience and shock-absorbing capacity. Chondrocytes within a normal articular cartilage are highly active and are largely responsible for the maintenance of the extracellular matrix in which they reside (36).

One of the earliest events in OA appears to be an increase in water content of the cartilage, implying failure in the elastic restraint of the collagen network due to structural alterations of collagen. Shortly afterwards, changes in PG composition occur, with an ultimate net loss of cartilage PGs from the extracellular matrix. This depletion suggests a failure in cytokine controlled matrix homeostasis, with a shift in the equilibrium between synthesis and degradation, favoring catabolic processes. In the early stages, there is an attempt to repair the loss of cartilage, indicated by an increase in the synthesis of the matrix macromolecules. However, the repair is often unsuccessful, possibly because of the altered composition and distribution of glycosaminoglycan as well as a change in its capacity to aggregate with hyaluronic acid.

In OA, we can additionally find an increased quantity of subchondral bone. Bone grows up into the calcified cartilage. Extensive bone remodeling deep to cartilage results in a more dense bone, with obliteration of intertrabecular spaces. Some investigators have suggested that this density makes the bone less able to attenuate joint loading forces or absorbance of

movement shocks, leading to accelerated damage to the overlying cartilage. Nevertheless, finite element analysis has suggested that even a marked increase in bone sclerosis would not markedly increase mechanical stress in the cartilage. The cause-and-effect relation of bone sclerosis and overlying cartilage loss is yet unclear. Animal studies have shown that an increased density of subchondral bone parallels and in some cases precedes covering cartilage loss. Although bone stiffness may cause cartilage damage, cartilage loss and bone sclerosis may be the consequences of mechanical overload. Because of the rapid remodelling of subchondral bone, the newly formed bone is not adequately mineralized and therefore not as strong and dense as it should be. At the joint margin endochondral ossification occurs and produces so called "osteophytes." Osteophytes may serve to stabilize joints. A variant of disease in which multiple large osteophytes exist is called hypertrophic OA. Synovial hypertrophy and fibrosis are seen in most joints affected by OA. Local synovitis affects 20% to 30% of diseased joints. Synovial involvement in OA may contribute to the disease by serving as the source of cytokines such as interleukin-1 that may turn off chondrocyte-mediated cartilage matrix synthesis. Furthermore, it triggers synthesis of degradating enzymes, by being a site for nociceptive fibers, and by secreting excess synovial fluid that makes the joint lax and therefore vulnerable to injury (49,76,116). Little evidence suggests that the synovial membrane in OA fails to provide sufficient nutrition to superficial layers of cartilage, or that the fluid secreted in OA is especially anti-adhesive and less lubricating than synovial fluid from undiseased joints.

TNF- α may drive the IL-1-induced cartilage degradation seen in rheumatoid arthritis (RA). However, the mechanisms that initiate and perpetuate similar cartilage degeneration in OA remain obscure. A quantitative rather than qualitative difference in cytokine profile is apparent, indicating that in OA, only few cells are recruited to cytokine production. This suggests that cartilage destruction takes place at far lower concentrations than originally believed. Maybe other processes are involved in OA and cytokines are merely indicators of disease activity (115,36,85,105).

2.3 Obesity - a state of chronic low-grade inflammation

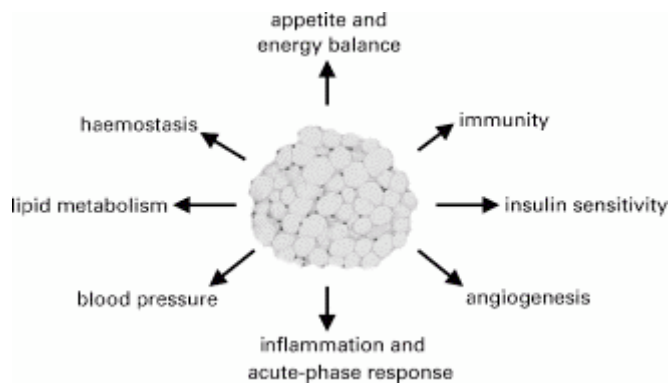


Image 3. Trayhurn, Bing C, Wood IS. Adipose tissue and adipokines—energy regulation from the human perspective. *J Nutr.* 2006 Jul;136(7 Suppl):1935S-1939S (108)

Obesity is now a considerable public health problem in most economically advanced countries and is associated with a reduction in life expectancy of up to 8 years. Moreover, it entails an increased risk of several major diseases, including type 2 diabetes, coronary heart disease, and certain cancers (such as breast and colon) (108). In the case of type 2 diabetes, the risk is particularly pronounced because of the likelihood to develop the disease by 10-fold once a BMI of 30 kg/m² is reached (108).

It is also known that visceral obesity accounts for a constellation of metabolic derangements, including insulin resistance, low HDL, elevated triglycerides, and raised blood pressure. This condition is summarized under the term metabolic syndrome (MetS), which significantly predicts cardiovascular disease risk. This condition is highly prevalent in the United States, affecting nearly 25% of all adults (116).

An association between obesity and the MetS has been clearly shown. Therefore, it became of great interest to find out, why and by what means adipose tissue might induce or contribute to these changes.

At first, white adipose tissue (WAT) was thought to serve one function only, which is to provide a capacity for triglyceride storage necessary for survival. Up to 90% of WAT is comprised of mostly lipid filled, round adipocytes, surrounded by loose connective tissue that is highly vascularized and innervated. It additionally contains macrophages, fibroblasts, adipocyte precursors, and various other cell types. The largest WAT depots are found in the subcutaneous region and around the viscera. The concurrent rise in insulin, glucose, and lipids during meals stimulates triglyceride formation and storage in liver and WAT. Conversely, the fall in insulin during fasting triggers glycogen breakdown and lipolysis through activation of the sympathetic nervous system and elevation of glucagon, epinephrine, and glucocorticoids. The latter maintain glucose supply to the brain and vital organs (1,49).

The conceptual transformation of adipose tissue from a passive organ of energy storage to an active participant in hormonal regulation of homeostatic systems occurred only recently. In 1994, adipose tissue was identified as the source of the hormone leptin, opening the door for a new era of research focused on adipocyte endocrinology (76,116). It is now accepted that adipose tissue plays a critical role in energy homeostasis, not only via storing triglycerides, but also by responding to nutrients, neural and hormonal signals, and secreting so called adipokines that play a part in the control of food intake, thermogenesis, immunity, and neuroendocrine function.

The diversity of the adipokines is considerable, in terms of both protein structure and function. The adipokines encompass classical cytokines, chemokines, proteins of the alternative complement system, the regulation of blood pressure, lipid metabolism, glucose homeostasis, and angiogenesis (108).

A very interesting finding was that amongst the adipokines, there are many inflammatory markers. Inflammation is one of the most important developing areas in obesity biology; the obese state was recognized recently as characterized by chronic low-grade inflammation (39). It is increasingly understood that the mild inflammatory state of obesity and particularly the production of inflammatory adipokines is important in the development of diseases associated with a high BMI (54). In particular, insulin resistance, type 2 diabetes, and atherosclerosis, as well as other components of the MetS, were causally linked to inflammation (108).

A central question is the origin of elevated circulating levels of inflammatory markers in obesity. There are three possibilities: at first, high levels of pro-inflammatory marker may reflect the production and release from organs other than adipose tissue, primarily the liver but also immune cells. The second explanation may be that WAT is secreting factors that stimulate the production of inflammatory markers from the liver and other organs; this is the case for CRP, whose hepatic production is under the control of IL-6, and therefore potentially derived from the expanded fat mass of the obese (119). The third possibility is that adipocytes themselves are the final source of these inflammatory markers. Furthermore, there is also, of course, the possibility of a combined production in adipose tissue and other organs (109).

Clear evidence for the expression and secretion of cytokines and chemokines in adipose tissue has been documented (i.e. TNF- α , transforming growth factor-beta, IL-6, IL-8, IL-10, MCP-1, and macrophage migration inhibitory factor) (108). Furthermore, the expression and release of

a number of inflammation related adipokines, including IL-6, TNF- α , haptoglobin, and leptin are increased in adipose tissue with obesity (1,50).

However, as pointed out before, adipose tissue is an inhomogeneous organ that is comprised of a variety of cell types, a fact that has prompted significant debate as to the true role of the adipocyte versus stromal/vascular and immune cells in secreting some of these endocrine and paracrine regulators. In fact, mature adipocytes seem to lack the storage vesicles and other structural cellular components usually associated with regulated release of secreted proteins by endocrine cells. In addition, the frequent use of pre-adipocyte cell lines to study adipocyte biology *in vitro* has increased this controversy because the functional characteristics (25) and transcriptional patterns of these multipotent cells are similar to immune cells. In fact, adipose precursor cells are considered to transdifferentiate into macrophages both *in vitro* and *in vivo* (22). Surprisingly, in these studies, increasing adiposity in mice correlated significantly with adipose tissue expression of a large cluster of genes characteristic for the expression from macrophages. Both adipocyte size and total body weight are strong predictors of the number of mature macrophages found within adipose tissue, a correlation being even stronger for visceral than for subcutaneous fat (26,114). These bone marrow-derived macrophages seem to invade fat in response to the release of soluble factors by adipocytes which increase the diapedesis of human blood monocytes across a layer of capillary endothelial cells. This effect can be mimicked by human recombinant leptin (26) since the leptin receptor is known to be expressed in endothelial cells from human adipose tissue. This strongly suggests that leptin could elicit endothelial cell activation and the infiltration of circulating blood monocytes into adipose tissue.

In obese animals, these macrophages tend to aggregate and form giant cells characteristic of chronic inflammatory disorders, suggesting that adipose tissue is a site of active inflammation (54). Gene expression studies on sorted cells from adipose tissue revealed that macrophages produce almost all TNF- α , whereas mature adipocytes secrete the majority of leptin and a similar IL-6 gene expression was found in macrophages, adipocytes, and non-macrophage stromal-vascular cells. Importantly, these studies suggest that macrophage invasion of fat and inflammation-related gene expression in adipose tissue may be a sentinel event, preceding the development of insulin resistance in these animals. Therefore, weight gain in mice is associated with infiltration of fat by macrophages and elaboration of proinflammatory signals from adipose tissue. Notably, these inflammatory changes are most marked within visceral fat.

This fat depot is associated with the greatest metabolic risk, and seems to precede other features of the MetS, including impaired glucose homeostasis (54,116,108,109).

Even though macrophages infiltrate fat as a result of obesity is surprising, interactions between adipose tissue and the immune system are well described, and various theories have been put forth to try to address the question of why such a link should exist. White adipose tissue and bone marrow share the same embryologic origin, the mesoderm, and pre-adipocytes are potent phagocytes that resemble, at least in part, macrophages in both morphology and patterns of gene expression (22). In addition, mature adipocytes share the ability to secrete cytokines and activate the complement cascade much like mononuclear immune cells.

The second argument for fat/immune system coordination is rooted in teleology. In *Drosophila*, the fat body is the organ that governs the innate immune system. Similarly in vertebrates, fat may be one of the crucial alarm systems that rouses the innate immune system and triggers the acute-phase response, which is the first line of defense against bacterial infection. The question of whether adipose tissue plays a significant role in controlling immune function in humans has yet to be answered conclusively.

Both, adipocytes and macrophages from adipose tissue secrete numerous hormones and cytokines that may contribute to the characteristic pathophysiologic changes observed in the MetS, and local inflammation within adipose tissue may be the sentinel event that causes systemic insulin resistance and systemic inflammation, two of the cardinal features of the MetS.

However, currently it is unclear why WAT releases pro-inflammatory cytokines and acute-phase proteins and why this event parallels the increase in fat mass. If the inflammatory response is primarily local to adipose tissue, at least in terms of its initiation, then the elevated circulating levels of inflammation-related products may reflect a spillover from the tissue, and the link with insulin resistance would be an incidental consequence.

A possible explanation is that it is a response to hypoxia in areas of the fat depots as the tissue mass increases during the development of obesity. The sequence of events might be that as the tissue expands, the vasculature (which is less extensive in WAT than in brown fat) is insufficient to maintain normoxia throughout the organ. Clusters of adipocytes then become relatively hypoxic, and an inflammatory response ensues. This could serve to increase blood flow and to stimulate angiogenesis, a similar mechanism to the tumor growth. A pivotal signal in the cellular response to hypoxia is hypoxia-inducible factor-1 (HIF-1). Hypoxia is

characteristic of tumors, where HIF-1 expression is increased, as well as in other disorders such as ischaemic heart disease. There is now evidence for the transcriptional activation of leptin through HIF-1 in response to hypoxia (3).

Another theory might be that, although adipocytes are capable of increasing in size, the cellular homeostasis and the secretory profile of larger adipocytes becomes altered and increasingly dysregulated compared with adipocytes of a smaller size. Although the total number of adipocytes is increased with increasing fat mass, the increased number and percentage of these large adipocytes may partially account for the inability of adipose tissue to function properly (88).

2.4 Adipokines

2.4.1 Leptin

The discovery of leptin in 1994 as a crucial factor involved in long-term regulation of food intake, body weight, energy expenditure and neuroendocrine functions has significantly broadened our understanding of the mechanisms underlying the development of obesity and its complications. Leptin is a 16 kDa protein and serum levels usually lie between 1-10 ng/ml (88,122) and its plasma concentrations rise in proportion to body fat mass (24,49). Although identified as a classic peptide hormone, the four α -helix domains in the folded structure make leptin most similar to cytokines such as IL-2 (122,24). Moreover, the leptin receptor bears significant homology to type 1 cytokine receptors; therefore, the hormone leptin is in many ways more appropriately identified as a cytokine (116). According to Loffreda et al. (66), leptin plays an important role in up-regulating inflammatory immune responses. They suggest that leptin enhances macrophage phagocytosis and the synthesis of proinflammatory cytokines such as TNF- α , IL-6 and IL-12. Leptin is mainly produced by adipocytes and in low levels by the gastric fundic epithelium, intestine, placenta, skeletal muscle, mammary epithelium, and brain (41,1).

Demonstration of the role of leptin in body weight homeostasis was provided by a mutation which occurred spontaneously in mice more than 50 years ago. The potent effect of recombinant leptin to reduce food intake, body weight and WAT mass in leptin-deficient mice brought the ultimate proof that the absence of functional leptin is responsible for the obese phenotype of *ob/ob* mice. Similarly, massively obese children with no functional leptin, are

currently successfully treated with a supplementation of recombinant leptin (35). Congenital leptin deficiency has been associated with hyperphagia, impaired thermogenesis, insulin resistance, hyperlipidemia, and central hypogonadism, all reversed by leptin treatment (35). Normally, leptin levels decline in concert with insulin during fasting and mediate the suppression of thyroid, growth, and reproductive hormones. This in turn stimulates appetite and inhibits sympathetic nerve activity, and thermogenesis (21). Obesity is associated with leptin production and high plasma leptin concentration. Soon after its discovery, it was realized that the rise in endogenous leptin or exogenous leptin treatment was unable to prevent weight gain in obese humans and rodents (41). This apparent "leptin resistance" may result from a decrease in brain transport or attenuation of leptin signaling in the hypothalamus and other central nervous system (CNS) targets (41,1). Beyond mutations in the receptor gene the molecular basis of leptin resistance are yet to be defined (49).

So far, three leptin receptors have been identified including a long, active form with 303 amino acid residues of its intracellular domain, and a short form with only 34 amino acids intracellularly. These forms have identical extracellular domains. A known mutation that causes obesity in mice (*ob/ob*) (70,122), just like leptin-deficiency, is a mutation of the leptin receptor gene. The long form is thought to be the functional receptor whereas the short form is conspicuously expressed in the choroid plexus, where it exhibits nanomolar affinity for leptin, and may function to regulate transport of serum leptin across the blood-brain barrier (70,76). Schwartz et al. (92) observed that leptin concentrations in the cerebrospinal fluid are correlated with plasma leptin levels. Again, these levels are correlated with the body mass index in humans. The long-form of the leptin receptor is associated with the production of neuropeptide Y (NPY) in the hypothalamus. It has further been reported that leptin administration caused an inhibition of NPY production. Given that NPY is a potent stimulant of hunger and an inhibitor of the sympathetic tone, at least a part of the function of leptin may be mediated through the action of NPY.

As obese individuals with the MetS generally have higher circulating leptin concentrations and seem to be resistant to the hypothalamic effects of leptin, their excess body weight is maintained. Although many of the effects of leptin result from a direct action on hypothalamic neurons, the long-form leptin receptor is also found on many tissues outside the central nervous system, including immune cells (116). Importantly, the mechanisms that are thought to contribute to hypothalamic leptin resistance (41) e.g., defective blood-brain barrier

transport, are unproved in peripheral tissues, and no studies have documented peripheral leptin resistance in obese individuals. Therefore, immune cells may well be subject to increased leptin effects in obese individuals, a signal that, on the basis of animal studies, may serve to activate the innate immune system and shift the cognate immune system toward a predominance of a proinflammatory Th1 population while reducing the regulatory Th2 phenotype. Although the immunomodulatory properties of increased leptin signalling are thought to be beneficial during acute infections in both rodents and humans, chronic elevations may be deleterious (13, 115).

Leptin, adiponectin and resistin have been detected in the synovial fluid (SYN) obtained from patients with OA (29,91). In experimental models, leptin may display pro- or anti-inflammatory effects in the joint depending on the immune response. In humans, Bokarewa et al. (11) suggested that intra-articular leptin may attenuate the erosive process in the joints during the course of rheumatoid arthritis. Leptin exhibited biological activity on chondrocytes with stimulation of anabolic functions through induction of growth factors synthesis and was shown to increase the effects of pro-inflammatory cytokines (82, 29, 87).

2.4.2 Adiponectin

Adiponectin is a 30-kDa protein that circulates at concentrations of 500–30,000 $\mu\text{g/l}$ in the blood stream, accounting for up to 0.01% of the total plasma protein. Circulating levels are approximately two to three times higher in females than in males (88, 89). Structurally, it consists of a collagenous tail and a globular head. Adiponectin shares strong sequence homology with C1q and the collagens typ VIII and X (122). The tertiary structure of the globular domain resembles TNF- α (1). Adiponectin monomers can form trimers, hexamers, and even higher-order multimers, and these oligomerizations influence its biological activity. Different properties have been ascribed to various recombinant or processed forms (globular head) of the protein and the actual bioactive form(s) has not yet been unequivocally determined (84, 76, 49).

Adiponectin was originally described as an adipokine exclusively expressed by adipose tissue, but in the meanwhile, it was found to be expressed by osteoblasts as well (9). In addition, hepatocytes, which normally do not express adiponectin, can synthesize adiponectin in response to IL-6 stimulation or tissue injury (117). Similarly, skeletal muscle cells are also able to express adiponectin when stimulated with inflammatory cytokines (28). These

findings indicate that adiponectin expression can be induced in cells other than adipocytes in the context of an inflammatory process (32).

As with leptin, also adiponectin plasma levels are higher in women than in men. But in contrast to leptin, adiponectin is reduced in obesity and increased in response to severe weight loss. Reduction of adiponectin has been associated with insulin resistance, dyslipidemia, and atherosclerosis in humans and rodents (1,15). A study in the obesity-prone Pima Indians suggests that individuals with high adiponectin concentrations are less likely to develop type 2 diabetes than those with low concentrations (64). Administration of recombinant adiponectin, either full length or in the form of its isolated globular head, exerts glucose lowering effects and ameliorates insulin resistance in mice-models of obesity or diabetes (49).

In addition, adiponectin has anti-atherogenic properties, as shown by its capacity to inhibit monocyte adhesion to endothelial cells and the macrophage-to foam-cell transformation *in vitro* (83). A recent study showed that adiponectin predominantly suppressed proliferation of myelomonocytic progenitors. It also inhibited mature macrophage functions, such as phagocytosis and cytokine production. These results suggest that adiponectin is an important regulator of the inflammatory response. Adiponectin is likely to regulate inflammatory responses negatively through at least 2 mechanisms: (1) suppression of mature macrophage functions and (2) inhibition of growth of macrophage precursors. The former is considered to play an important role in the control of early responses of inflammation, and the latter may act in late events of inflammation to prevent immune responses from continuing chronically (118). TNF- α and IL-6 are potent inhibitors of adiponectin expression and secretion in human WAT biopsies or cultured adipose cells. IL-8 was found to be only a mild inhibitor (49,33,15). This suggests that endogenous cytokines may inhibit adiponectin, which could be of importance for the association between cytokines (i.e.IL-6) and insulin resistance and atherosclerosis.

There are two adiponectin receptors. The first, named AdipoR1 is specifically expressed in muscle. The second, AdipoR2 is specifically expressed in the liver. The muscle receptor responds to adiponectin globular domain fragments. On the other hand, the liver receptor, is only stimulated by full-length adiponectin oligomers (76). Adiponectin receptors 1 and 2 contain seven-transmembrane domains, but are structurally and functionally distinct from G protein-coupled receptors.

Whether adiponectin is able to enter the brain is controversial. Iodinated globular adiponectin does not cross the brain-blood barrier in mice. Nonetheless, murine cerebral microvessels express AdipoR1 and R2, which are up-regulated during fasting. Globular adiponectin reduced the release of IL-6 from brain endothelial cells, providing a potential mechanism of action.

Adiponectin, in particular the trimeric form, has been shown in human cerebrospinal fluid (100).

2.4.3 IL-6

The human IL-6 is a protein consisting of 186 amino acids. It is synthesized as a precursor protein of 212 amino acids. At least five different molecular forms of IL-6 with molecular masses from 21 to 28 kDa are expressed in monocytes. This difference is derived from posttranslational alterations such as glycosylation and phosphorylation. The IL-6 receptor consists of two subunits: the alpha chain, a 80kDa transmembrane glycoprotein which binds IL-6 with low affinity, and the beta chain, a 130kDa transmembrane glycoprotein which forms the high-affinity signal transducing complex. In healthy individuals, serum IL-6 is usually <10 pg/mL (2,121). It is secreted by many cell types, including immune cells, fibroblasts, endothelial cells, skeletal muscle and adipose tissue (89).

Most cytokines function predominantly as paracrine or autocrine factors. However, IL-6 is an endocrine cytokine, meaning that most cellular targets of this cytokine are distant from the site of release and the effects of IL-6 are correlated with its serum concentrations. Within adipose tissue, both adipocytes and macrophages secrete IL-6 (114), and studies measuring arteriovenous increases of serum IL-6 concentration have clearly shown the secretion of IL-6 from adipose tissue depots. Adipose tissue accounts for up to 30% of circulating IL-6 concentrations in humans (77). Like leptin, production of IL-6 by adipose tissue rises with increasing adiposity, and circulating IL-6 concentrations are highly correlated with percentage of body fat and with insulin resistance (8, 49,116,89).

Although IL-6 is a highly pleiotropic cytokine, with hormonal effects on many tissues, the effects on the liver, bone marrow, and endothelium are thought to be most significant in contributing to the metabolic effects of obesity and it appears likely that the elevated adipose expression of IL-6 in the obese state could be essential for signalling between adipose tissue and distant organs (50). Circulating IL-6 is the single most important factor controlling the hepatic acute-phase response, the rapid, coordinated physiologic reaction to tissue damage or infection designed to recruit host defense mechanisms, eliminate damaged cells, contain pathogens, and initiates tissue repair.

Moreover, IL-6 in combination with its soluble receptor sIL-6R α , dictates the transition from acute to chronic inflammation by changing the nature of leucocyte infiltrate. In addition, IL-6 exerts stimulatory effects on T- and B-cells, thus favoring chronic inflammatory responses

(42). The main shift from acute to chronic inflammation is the recruitment of monocytes to the area of inflammation. At the beginning of acute inflammation, IL-6 mediates the acute phase responses. When its activity as a proinflammatory cytokine persists, the acute inflammation turns into a chronic inflammation that includes immune responses. In chronic inflammation, IL-6 has a detrimental role that favours mononuclear cell accumulation at the site of injury, through continuous MCP-1 secretion, angioproliferation and anti-apoptotic functions on T-cells. This may increase serum levels of IL-6 and provide the basis for the amplification of a chronic inflammation. Plasmacytosis and hyperplasia of synovial cells in the joints of patients with rheumatoid arthritis (RA) are typical examples of a chronic inflammatory reaction. In autoimmune diseases, IL-6 not only maintains inflammation but also modifies the immune responses. Levels of circulating IL-6 are elevated in several inflammatory diseases including RA, systemic juvenile idiopathic arthritis, systemic lupus erythematosus, ankylosing spondylitis, psoriasis and Crohn's disease (42). However, recent reports of high levels of IL-6 production within the joint suggest a role in OA. In a canine model, IL-6 concentration was found to be greater in synovial fluid from the damaged, compared with the control limb, indicating an injury-induced production (115).

In the CNS, IL-6 is a powerful catabolic agent that leads to decreased food intake and increased energy expenditure, based on numerous studies involving the administration of IL-6 into the cerebral ventricles. The expression and release of IL-6 by neurons and glial cells seems to be essential for the effects of this cytokine on energy balance, but it remains unclear to what extent central IL-6 production is controlled by circulating IL-6 in the serum, although transport mechanisms seem to exist to deliver IL-6 across the blood-brain barrier. Importantly, mice with a genetic deletion of IL-6 develop adult-onset obesity, suggesting that this cytokine is involved in the chronic physiologic regulation of energy balance and that decreased IL-6 signalling is associated with weight gain. Therefore, if IL-6 secretion by adipose tissue contributes to energy homeostasis through an endocrine action on the CNS, then one could invoke a state of obesity-induced IL-6 resistance, much as described for the effects of obesity on leptin and insulin signalling. This supposition also suggests the possibility that increased adipose-tissue IL-6 secretion associated with obesity may be a regulatory mechanism attempting to correct excess body weight and achieve negative energy balance, as hypothesized for obesity-related increases in leptin. The systemic inflammation resulting from IL-6 effects on liver and endothelium therefore could be an unintended consequence of appropriately elevated IL-6 levels in the face of obesity and central IL-6 resistance (116).

2.4.4 IL-8

Four types of differentially processed forms of IL-8, consisting of 69, 72, 77, and 79 amino acids, are known. Among these variants, the 72 amino acid form is predominantly released and exerts its greatest activity on neutrophils. Normal serum levels are below 50 pg/ml (121). IL-8 binds to two types of receptors, CXCR1 and CXCR2, which both belong to the G protein-coupled seven transmembrane receptor superfamily. It is produced by phagocytes and mesenchymal cells exposed to inflammatory stimuli (i.e. interleukin-1 or TNF) and activates neutrophils; inducing chemotaxis, exocytosis and the respiratory burst. In vivo, IL-8 elicits a massive neutrophil accumulation at the site of injection. Five neutrophil-activating cytokines similar to IL-8 in structure and function have been identified recently. IL-8 and the related cytokines are produced in several tissues upon infection, inflammation, ischemia, trauma etc., and are thought to be the main cause of local neutrophil accumulation. These stimuli induce three main responses in neutrophils: 1) shape change and directional migration, 2) exocytosis of storage proteins, and 3) the respiratory burst (6,47). The shape change reflects the activation of the contractile system and enables the neutrophils to adhere to endothelial cells and to migrate. Exocytosis leads to the release of soluble storage proteins and the remodeling of the neutrophil plasma membrane by fusion with the storage organelles. IL-8 triggers the exocytosis of specific granules and secretory vesicles. The respiratory burst is characteristic for stimulated phagocytes. Like other chemoattractants IL-8 elicits the rapid and transient activation of the NADPH-oxidase, leading to superoxide and H₂O₂ formation (6).

Many different cells have the ability to produce IL-8 upon stimulation. The expression of IL-8 mRNA and the release of the biologically active cytokine were observed in endothelial cells, fibroblasts from different tissues, keratinocytes, synovial cells, chondrocytes, several types of epithelial cells as well as some tumor cells. Interestingly, even neutrophils can synthesize IL-8, and may thus intensify their own recruitment to sites of inflammation. IL-1 and TNF are clearly the most important stimuli, since they were found to induce IL-8 expression and secretion in all cells studied so far (6,122).

IL-8 has been shown to be produced and released from human isolated adipocytes and whole adipose tissue cultures in a regulated manner (14). Besides its association with a number of different inflammatory processes, IL-8 has also been implicated in the pathogenesis of atherosclerosis and coronary heart disease (45). Plasma levels of IL-8 have been found to be

significantly increased in patients with both type 1 and type 2 diabetes compared with healthy subjects (121). It has been reported that circulating IL-8 correlates with measures of adiposity and insulin sensitivity, suggesting an involvement of IL-8 in some of the obesity-related health complications (14).

2.4.5 TNF- α

TNF- α is an inflammatory cytokine and has chemotactic effects on monocytes and neutrophils. Originally, the search for the cause of hemorrhagic necrosis in tumors led to the discovery of TNF. This cytokine is a protein that exists in a soluble (157 amino acids) and a transmembrane form (233 amino acids) and mediates its action through the two distinct receptors p60 and p80. TNF- α is primarily produced by macrophages in response to various inflammatory stimuli. It was the first adipose secreted product proposed to represent a molecular link between obesity and insulin resistance. Indeed, TNF- α is overexpressed in WAT in obesity and decreases with weight loss and improvement of insulin sensitivity (50,49). However, it is not clear that WAT contributes to circulating levels of TNF- α in humans, which suggests an autocrine-paracrine mode of action of the cytokine rather than an endocrine effect.

Within adipose tissue, macrophages account for nearly all TNF- α production (114,109,116) but there are other studies hypothesizing that both adipocytes and stromavascular cells as well as macrophages produce TNF- α (89).

Therefore, it remains unclear whether TNF- α secretion from adipose tissue directly accounts for the elevated serum TNF- α concentration seen in obesity. Nonetheless, TNF- α clearly may have an important role as a paracrine factor in the inflammatory response triggered by obesity independent of circulating concentration of the cytokine.

More recently, another potential mechanism whereby TNF- α influences insulin resistance has been identified. Adiponectin secretion by adipocytes is potently reduced by TNF- α signalling, and this hormone seems to be a crucial mediator of insulin sensitivity, thereby potentially explaining how paracrine effects of TNF- α within fat could cause systemic insulin resistance (122,61).

Furthermore, stimulation of neutrophils with TNF- α induces phagocytosis, adherence of these cells to endothelial cells, and generation of free radicals of oxygen-superoxide anion, and hydrogen peroxide. Stimulation of cultured human endothelial cells with TNF induces procoagulant activity. The cytokine also induces synthesis of the inflammatory cytokine IL-8 and other chemotactic cytokines that regulate migration, degranulation, and respiratory burst

response of neutrophils. TNF- α induces the synthesis of other chemokines, such as MCP-1, that promotes accumulation of monocytes at the site of inflammation. TNF- α is involved in remodeling of connective tissue. From fibroblasts and synovial cells TNF- α induces the release of collagenase and other matrix metalloproteases enzymes. It inhibits collagen synthesis in bone and in explanted cartilage it reduces the content of alkaline phosphatase and induces resorption of proteoglycan.

Usually, TNF- α is undetectable in the serum of normal human subjects. Low levels of TNF- α in the serum have, however, been detected in patients with gram-negative bacterial infections, fever, or cancer. Extensive research within the last 15 years has revealed that although TNF- α is required for protection against bacterial infection, it is involved in cell growth modulation, viral replication, immune system regulation, septic shock, autoimmune diseases, rheumatoid arthritis, inflammation, and diabetes. Agents that can block TNF- α action, such as thalidomide, soluble TNF- α receptors, and anti-TNF- α antibodies, have been approved for human use for autoimmunodeficiency disease syndrome (AIDS), rheumatoid arthritis, and inflammatory bowel disease, respectively.

A recent elegant hypothesis suggested that in obese rats TNF- α production from the fat cuff around the arteriole inhibits insulin-stimulated nitric oxide synthesis and results in unopposed vasoconstriction - a mechanism termed 'vasocrine' signalling (120). These findings suggest a homology between vasoactive periarteriolar fat and visceral fat, which may explain relationships among visceral fat, insulin resistance and vascular disease.

2.4.6 MCP-1

There are several monocyte chemoattractant proteins (MCPs). All of them are also chemoattractants for monocytes and activated T cells. Some MCPs also attract eosinophils. Their expression pattern suggests that these chemokines may play an important role in human diseases that are characterized by mononuclear or eosinophilic cell infiltration.

MCP-1 is a monomeric protein consisting of 76 amino acids that is derived by proteolytic cleavage of a 99-amino-acid precursor. MCP-1 is typically secreted in two predominant forms with apparent molecular weights of 9 and 13kDa. MCP-1 is highly specific in their receptor usage, binding only to human or murine CCR2 with high affinity (122).

MCP-1 in particular is a major monocyte chemoattractant, and stimulates both chemotaxis of monocytes and several cellular events associated with chemotaxis, including calcium flux and expression of integrins. It is also a weak inducer of cytokine expression in monocytes. MCP-1 has a restricted target cell population consisting of monocytes, and basophils. In addition, MCP-1 has been shown to stimulate a subset of CD4⁺ and CD8⁺ lymphocytes and memory T-lymphocytes *in vitro*. Besides chemotaxis, MCP-1 is one of the most potent inducers of histamine release from basophils. This cytokine is considered to be crucially important in the pathogenesis of the following diseases: Idiopathic pulmonary fibrosis, bacterial-induced gingivitis, atherosclerosis, delayed-type hypersensitivity reactions in the skin, tumor (melanoma), rheumatoid arthritis and renal ischemia (47,122).

Concerning WAT, it has recently been shown that adipocytes secrete a factor that up-regulates adhesion molecules on endothelial cells and increases the chemotaxis of monocytes. These effects can be mimicked by leptin (26). The secretion of MCP-1 from adipose tissue and isolated fat cells points toward a specific role of this chemokine within the tissue. By secretion into the local circulation it could cause recruitment of monocytes and thus contribute to the observed macrophage infiltration of adipose tissue in obesity. The recruitment of monocytes could be either a direct interaction with these cells or a positive stimulation of leptin, which can act as a recruitment factor for monocytes and up-regulate adhesion molecules in endothelial cells (26,27).

Increased secretion and adipose tissue mRNA levels of MCP-1 in human obesity have been reported. But, it is still controversially discussed whether there is an increase in the circulating MCP-1 concentration in obese (27).

3. Patients and Methods

3.1 Patients

37 patients were included in this study (ten male and 27 female) suffering from OA and therefore underwent total knee arthroplasty. The IFP was excised, as was a subcutaneous adipose tissue sample at the proximal end of the incision. A sample of synovial membrane was also obtained from 22 patients. In many cases, synovial fluid (SYN) was bloody. Therefore, a sample of synovial fluid could only be obtained from 23 patients. The mean age of the donors was 71.6 ± 9.5 yr, and their average body mass index (BMI) was 28.7 ± 4.5 kg/m². The patients also suffered from a variety of co-morbidities (cardiovascular diseases, type 2 diabetes mellitus, lipometabolic disorders, osteoporosis, renal insufficiency, or Parkinson's disease). Patients with acute infections or consuming diseases were excluded from this study. Three patients were diagnosed with rheumatoid arthritis. The patients gave their informed consent to the procedure.

3.2 Isolation and Culture of Adipocytes

The samples were immediately transported to the laboratory in DMEM containing 20 µg/ml gentamicin. The samples were not cooled during the transport. In the laboratory, the synovial fluid was immediately stored at -80° C. Synovial membrane, IFP and subcutaneous fat (SCF) from the thigh were placed in PBC-buffer for further dissection. A piece of synovial membrane (usually between 100-150 mg) was cut into fine pieces and incubated in 5.4 ml DMEM/F-12 standard medium containing 17.5 mM glucose without fetal bovine serum (FBS) supplemented with 20 µg/ml gentamicin for 24 hrs.

Then the weight of the IFP was measured. Connective tissue and visible blood vessels were removed from the IFP and the SCF with scissors. For preparation of mature adipocytes, the adipose tissue was minced and digested in Krebs-Ringer-phosphate buffer (KRP; 154 mM NaCl, 100 mM NaH₂PO₄, 154 mM KCl, 154 mM MgSO₄, 110 mM CaCl₂, pH 7.4) containing 100 U/ml collagenase and 4% BSA for 60 min at 37° C in a shaking water bath. After this step, the undigested tissue was removed by filtration through a nylon mesh with a pore size of 250 µm (VWR, Darmstadt, Germany). The floating adipocytes were washed three times with KRP containing 0.1% BSA.

Aliquots of each sample were used to determine mean cell diameters. The assessment was based on the measurement of 100 cells under the microscope. The cell volume was calculated from the radius using the formula $\frac{4}{3} \cdot r^3 \cdot \pi$ and subsequently 1 million cells were incubated in 5.4 ml DMEM/F-12 standard medium containing 17.5mm glucose without fetal bovine serum supplemented with 20 $\mu\text{g}/\text{ml}$ gentamicin for 24 hrs. After 24 hrs, the conditioned media were immediately stored at -80°C for further analysis. Adipocytes were collected and were also stored immediately at -80°C .

3.3 Measurement of Adipokine Release

The concentrations of adiponectin and leptin in the cell culture media were measured using the quantitative sandwich enzyme immunoassay technique from R&D Systems (Wiesbaden, Germany). A monoclonal antibody specific for leptin or adiponectin had been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any leptin/adiponectin present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for leptin or the adiponectin globular domain was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of leptin/adiponectin bound in the initial step. The color development was stopped and the intensity of the color was then measured.

The concentrations of IL-6, IL-8, TNF- α , and MCP-1 granulocyte were measured with a multiplex bead-based assay from Bio-Rad. This system is built around 3 core technologies. The first is the family of fluorescently dyed microspheres (beads) to which biomolecules are bound. The second is a flow cytometer with two lasers and associated optics to measure biochemical reactions that occur on the surface of the microspheres. The third is a high-speed digital signal processor that efficiently manages the fluorescent output.

The multiplex technology uses up to 100 color-coded bead sets, each of which can be conjugated with a specific reactant. Each reactant is specific for a different target molecule. This cytokine assay is designed in a capture sandwich immunoassay format. Antibody specifically directed against the cytokine of interest is covalently coupled to color-coded 5.6 μm polystyrene beads. The antibody-coupled beads are allowed to react with a sample containing an unknown amount of cytokine or with a standard solution containing a known

amount of cytokine. After performing a series of washes to remove unbound protein, a biotinylated detection antibody specific for a different epitope on the cytokine is added to the beads. The result is the formation of a sandwich of antibodies around the cytokine. The reaction mixture is detected by the addition of streptavidin-phycoerythrin, which binds to the biotinylated detection antibodies. The constituents of each well are drawn up into the flow-based suspension array system, which identifies and quantifies each specific reaction based on bead color and fluorescence. The magnitude of the reaction is measured using fluorescently labelled reporter molecules associated with each target protein. Unknown cytokine concentrations are automatically calculated using a standard curve derived from a recombinant cytokine standard. The amount of protein in the medium from the incubations was either normalized for 1 million cells or calculated per 100 mg of tissue.

3.4 Statistical analysis

SPSS was used for statistical analysis. Spearman's test and Mann-Whitney test were applied. The Spearman's test is a non-parametric measure of correlation. It assesses how well an arbitrary monotonic function could describe the relationship between two variables, without making any assumptions about the frequency distribution of the variables. The Mann-Whitney test is a non-parametric test for assessing whether two samples of observations come from the same distribution. The null hypothesis is that the two samples are drawn from a single population, and therefore that their probability distributions are equal. It requires the two samples to be independent, and the observations to be ordinal or continuous measurements, i.e. one can at least say, of any two observations, which is the greater. The threshold for significance was set at $p < 0.05$ and $p < 0.01$.

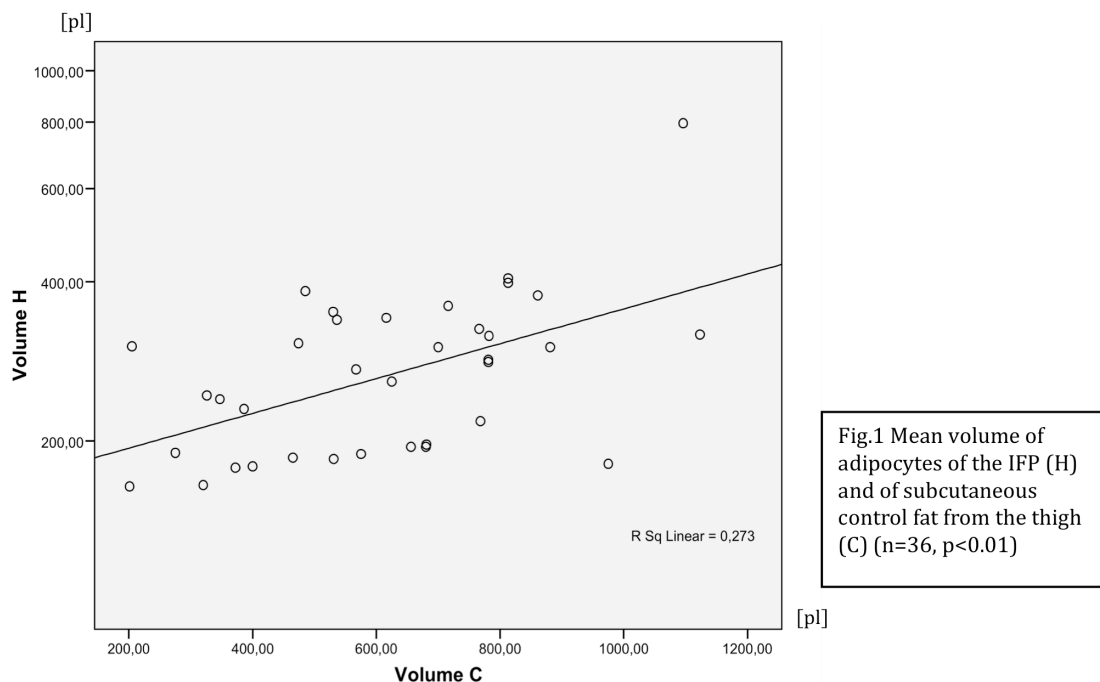
4. Results

4.1 Weight IFP

The mean weight of the IFP was 12.2 ± 4.4 g. There were no significant correlations between weight of the IFP and BMI, age or volume of the adipocytes from the IFP. However, there was a significant correlation between weight of the IFP and the adiponectin-production in subcutaneous fat from the thigh (C) (see p46, Figure 20). It must be mentioned, that re-operated patients were excluded from the analysis, as IFP might already been removed during the first operation.

4.2 Adipocyte volume of adipose tissue samples

Mean volume of adipocytes from the IFP was 282.2 ± 113.6 pl and mean volume of adipocytes from control fat was 614.1 ± 231.2 pl. Statistically, fat cell from the IFP are therefore significantly smaller than from control fat ($p < 0.01$). Furthermore, there was significant correlation between adipocyte volume of H and adipocyte volume of C ($r = 0.5$, $p < 0.01$) (Figure 1).



4.3 Secretion of adipokines from IFP (H), control fat (C) and synovial membrane (S), and correlations with anthropometric parameters

Adipocytes were prepared and cultured as described under Materials and Methods. Conditioned media were measured with commercially available ELISA-assays according to the instructions of the manufacturer. In the following, the concentration of adipokines released after 24 hrs of incubation were either normalized for 1 mio. cells or for 100 mg tissue.

4.3.1 IL-6

The amount of IL-6 released from 1 mio. cells of H varied greatly and was between 142.5 pg/ml and 41,316.3 pg/ml. The median value was 2,001 pg/ml. For control cells from C the amount of IL-6 released from 1 mio. cells of C was between 170.9 pg/ml and 38,665 pg/ml. The median value was 941.7 pg/ml (Figure 2). There were no statistically significant gender-specific differences in the IL-6 release from H and C. Furthermore, no correlations were detected between IL-6 release and BMI, IFP-Weight or adipocyte-volume, but there was a significant negative correlation between age and IL-6-release from H ($r=0.398$; $p<0.05$) (Figure 4).

Calculated per the same amount of tissue IL-6 release from 100 mg of C, H and S showed the following. Medium levels for IL-6 were between 31.2 pg/ml and 2,4161 pg/ml secreted from H fat. The median value was 941.7 pg/ml. C fat released between 90.9 pg/ml and 3,761 pg/ml and the median value was 621.3 pg/ml. The IL-6-release from S varied between 4,345 pg/ml and 40,000 pg/ml and the median value was 17,978 pg/ml (Figure 3).

There were no statistical significant gender-specific differences and no correlations between BMI, age, IFP-Weight, adipocyte-volume and IL-6 release from 100 mg tissue of H, C or S.

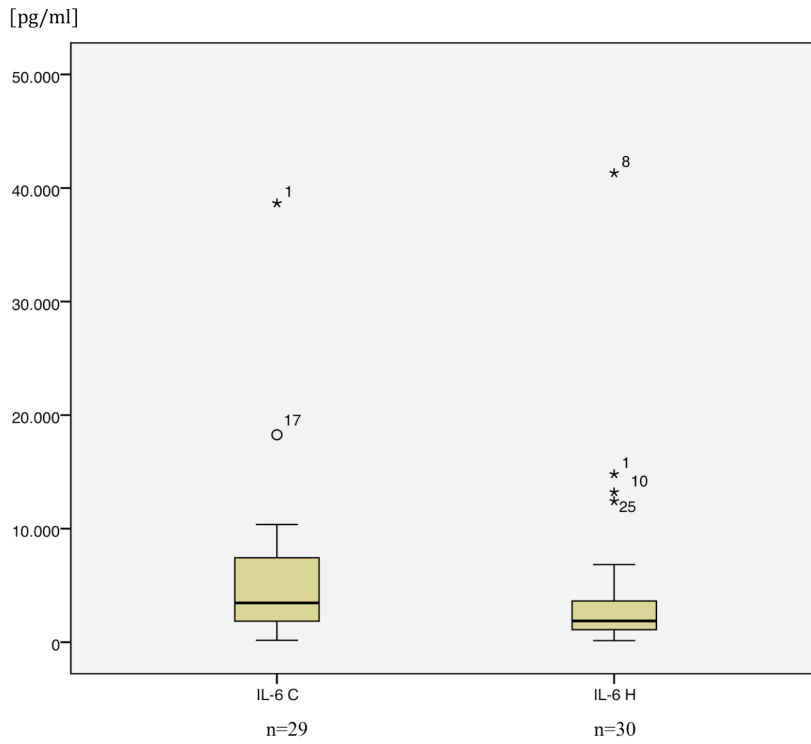


Fig.2: IL-6 release from 1 mio. adipocytes of subcutaneous adipose tissue of the thigh (C) and 1 mio. cells of the IFP (H). Results are given as mean values \pm SEM. (p=n.s.)

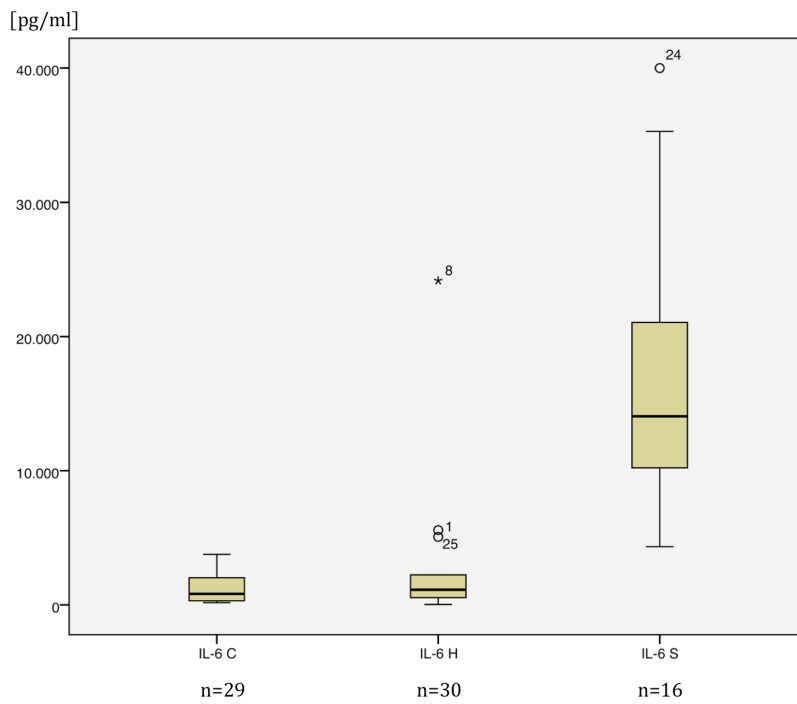
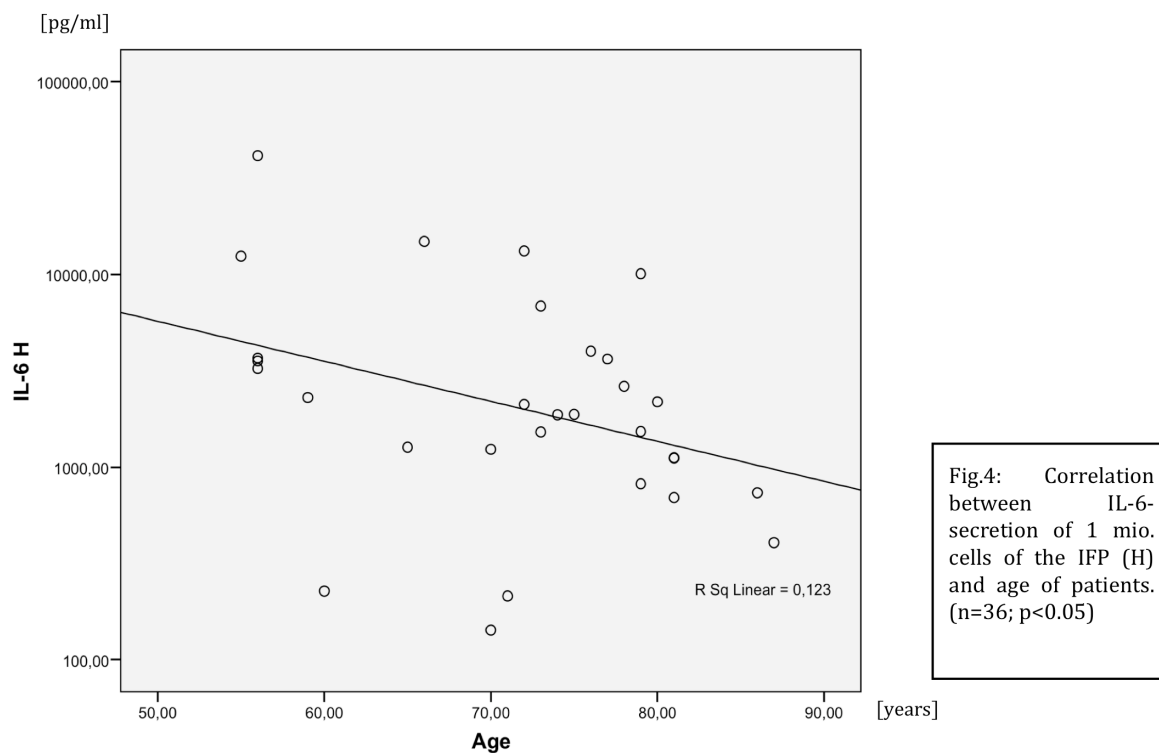


Fig. 3: IL-6-release from 100mg tissue of subcutaneous fat (C), the IFP (H) and synovial membrane (S). Results are given as mean values \pm SEM. (p=n.s.)



4.3.2 IL-8

One million adipocytes of H released between 146.4 pg/ml and 24,338 pg/ml of IL-8 with a median value of 3,351 pg/ml. The IL-8 production of C lay between 292.1 pg/ml and 66,662 pg/ml with the median value being 1,0014 pg/ml (Figure 5). No gender-specific differences or correlations between BMI, IFP-weight, adipocyte volume and IL-8-release of H or C were found, but there was a significant negative correlation between the age of patients and the IL-8-production of H. ($r=0.393$; $p<0.05$) (Figure 7).

Calculation per 100mg tissue revealed IL-8 production of the IFP ranging between 54.3 pg/ml and 14,473 pg/ml with a median value of 1,635 pg/ml. The IL-8 release from C lay between 108.5 pg/ml and 11,273 pg/ml and the median value was 1,719 pg/ml. One hundred mg of synovia produced between 2,092 pg/ml and 35,000 pg/ml IL-8 and the median value was 15,510 pg/ml (Figure 6).

Again, no statistically significant gender-specific differences or correlations were found between BMI, age, IFP-Weight, adipocyte-volume and IL-8-release of H, C or S.

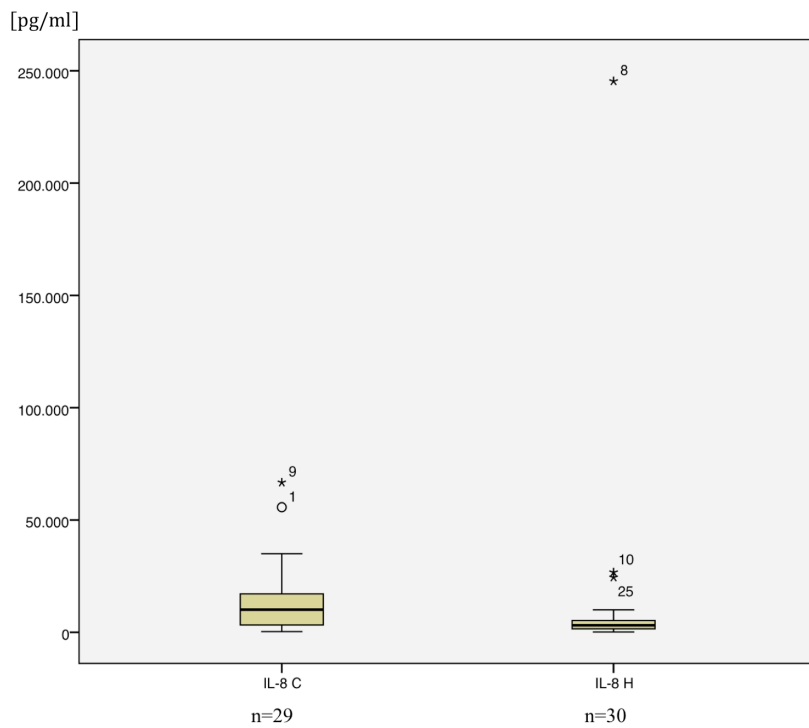


Fig. 5: IL-8-release from 1 mio. adipocytes from subcutaneous adipose tissue (C) and 1 mio. cells from the IFP (H). Results are given as mean values \pm SEM (p=n.s.).

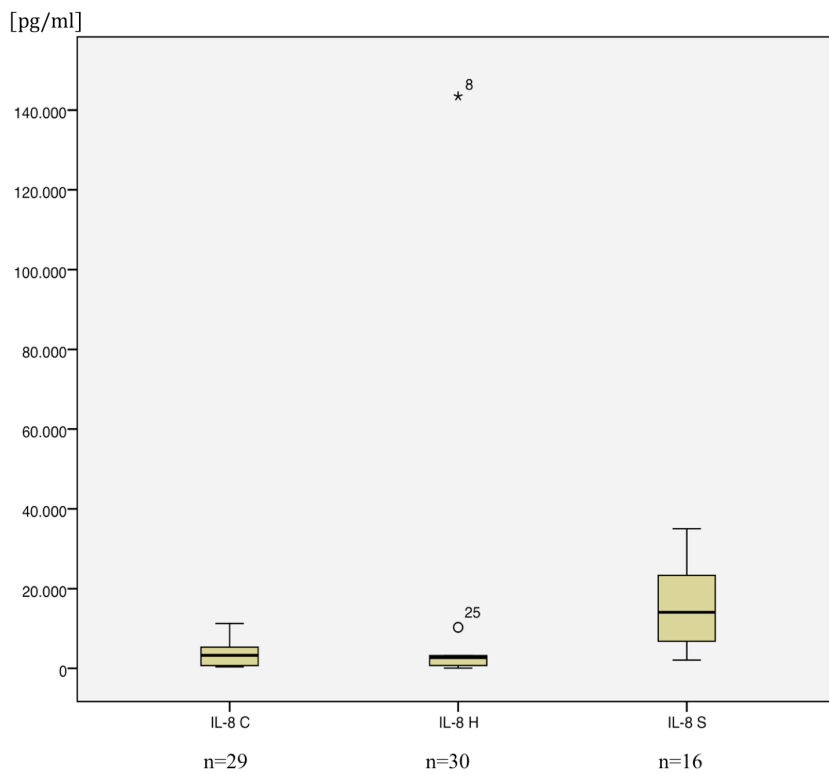
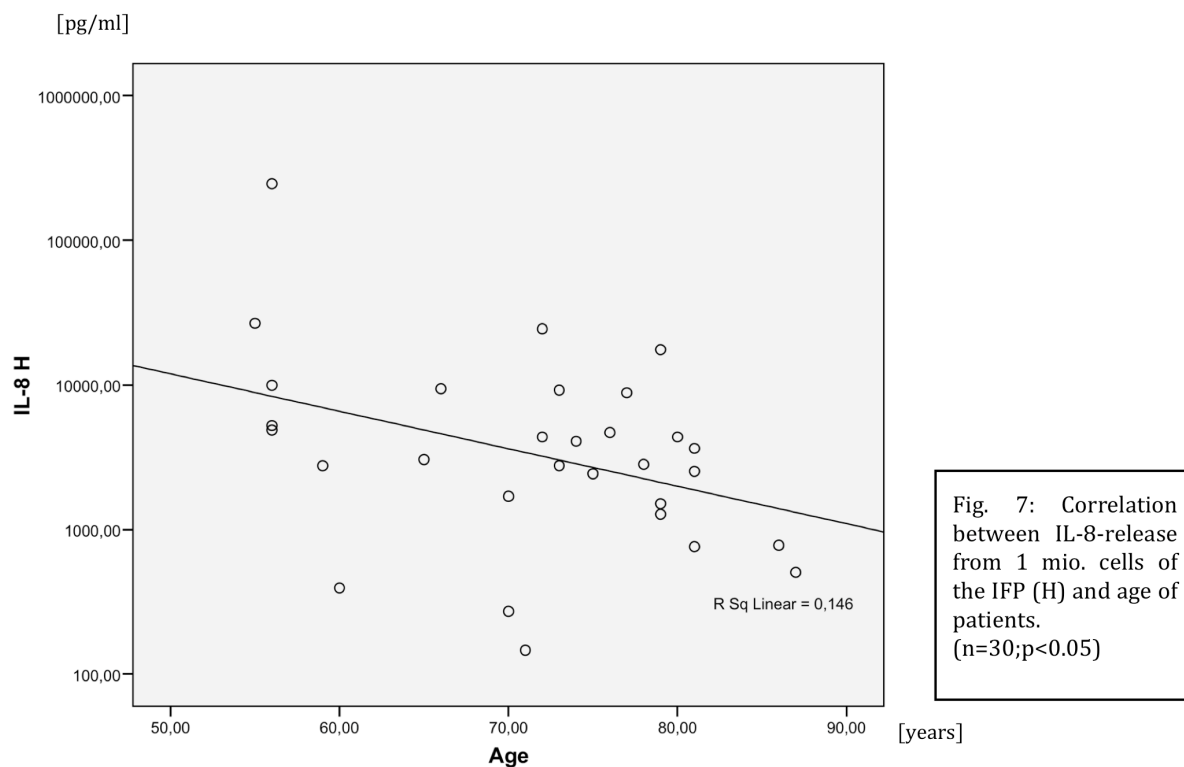


Fig. 6: IL-8-release of 100mg tissue from subcutaneous fat (C), the IFP (H) and synovial membrane (S). Results are given as mean values \pm SEM (p=n.s.)



4.3.3 TNF- α

TNF- α released from 1 million cells of H was between 0.29 pg/ml and 72.7 pg/ml and the median value was 8.3 pg/ml. C produced between 0.29 pg/ml and 109.2 pg/ml. The median value was 13.1 pg/ml (Figure 8). No statistically significant gender-specific differences or correlations between BMI, age, IFP-Weight, adipocyte-volume and TNF- α -release of H or C were found.

One hundred mg of tissue from the IFP secreted between 0.1 pg/ml and 36.4 pg/ml TNF- α and the median value was 3.4 pg/ml. The TNF- α release from C lay between 0.06 pg/ml and 15.5 pg/ml and the median value was 3.1 pg/ml. One hundred mg of synovial membrane produced between 0.6 pg/ml and 74.2 pg/ml TNF- α and the median value was 32.8 pg/ml (Figure 9). No statistically significant correlations between BMI, age, IFP-Weight, adipocyte-volume and TNF- α -release of H, C or S were found. There was however a significant difference of the TNF- α production of H between men and women ($p < 0.05$) (Figure 10).

There was moreover a statistically significant correlation between the TNF- α production of 1 mio. cells of S and H ($r = 0.43$, $p < 0.01$) (also per 100mg tissue of S and H, $r = 0.43$, $p < 0.01$) (Figures 11 and 12) as well as a correlation between TNF- α production of 1 mio. cells of S and C ($r = 0.53$, $p < 0.05$) (also per 100mg tissue of S and C, $r = 0.6$, $p < 0.05$) (Figures 13 and 14).

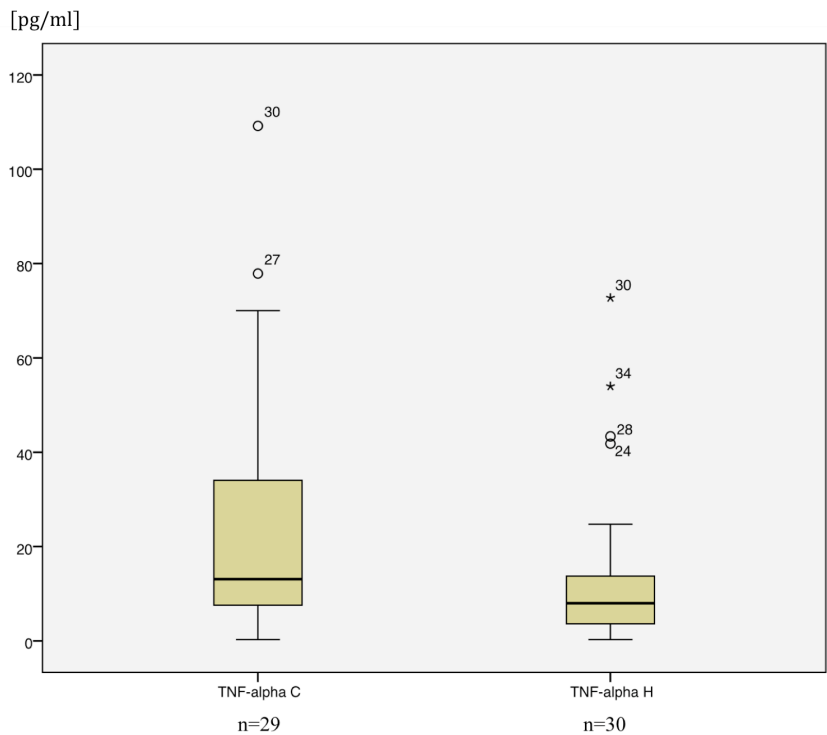


Fig. 8 TNF- α -release from 1 mio. adipocytes of subcutaneous fat (C) and from 1 mio. cells of the IFP (H). Results are given as mean values \pm SEM (p=n.s.)

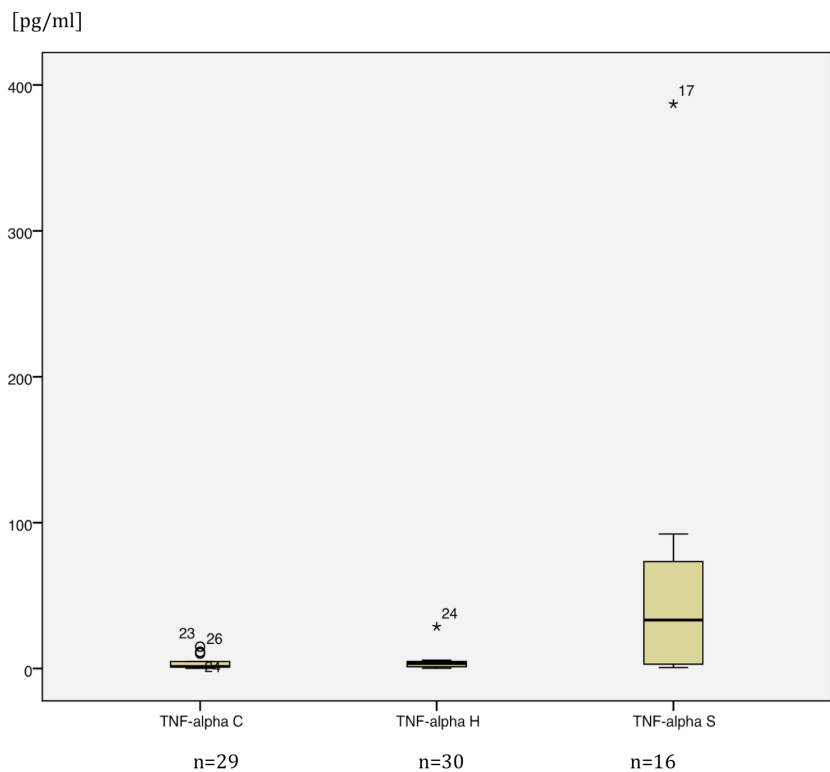


Fig. 9: TNF- α -release from 100mg tissue of subcutaneous fat (C), the IFP (H) and synovial membrane (S). Results are given as mean values \pm SEM (p=n.s.).

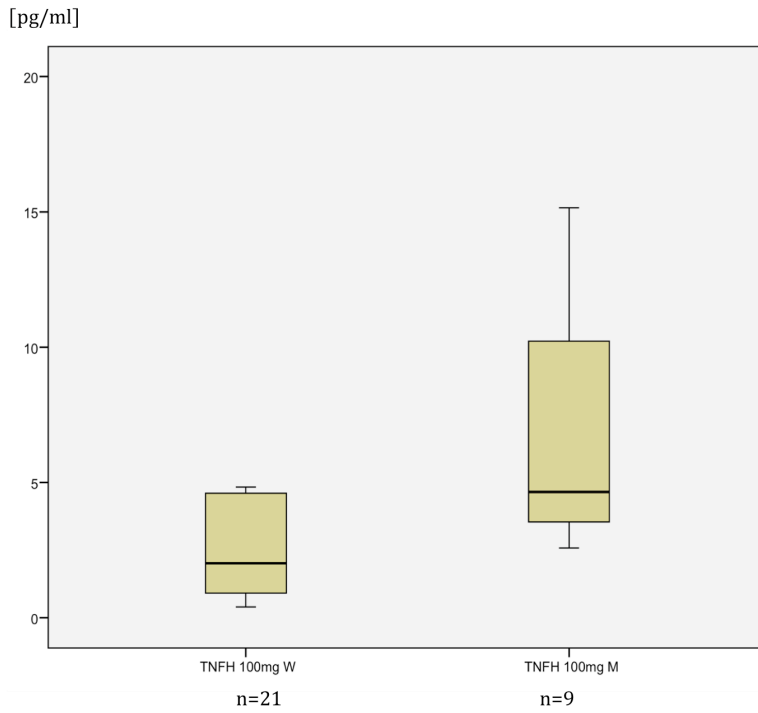


Fig. 10: TNF- α -secretion of 100mg tissue of the IFP (H) in Women (W) and in Men (M). Results are given as mean values \pm SEM ($p < 0.05$).

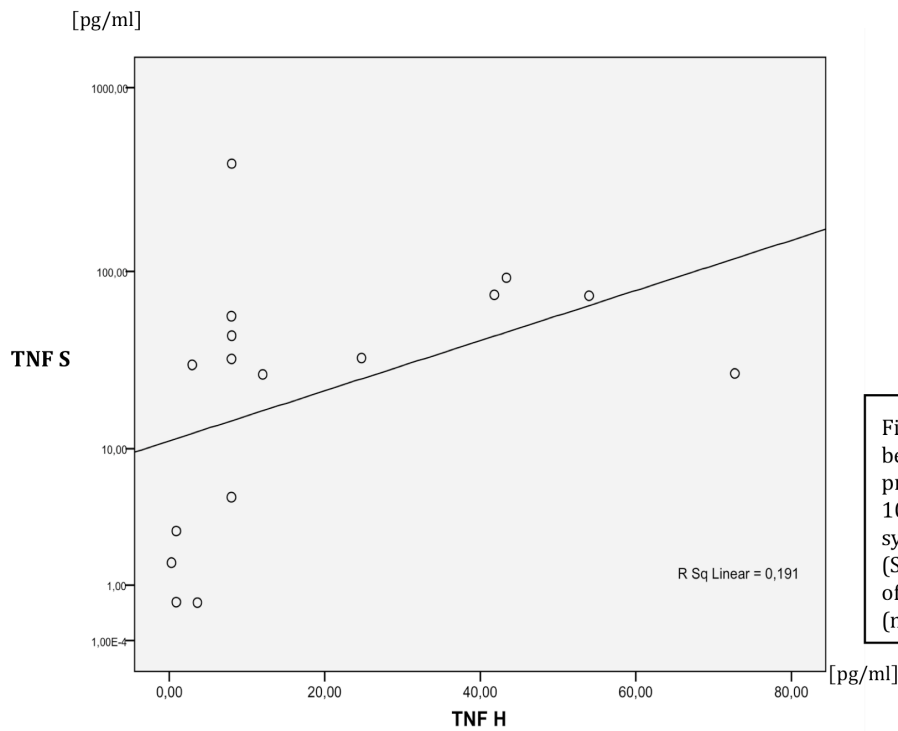


Fig. 11 Correlation between TNF- α -production in 100mg tissue of synovial membrane (S) and 1 mio. cells of the IFP (H). ($n=16; p < 0,01$)

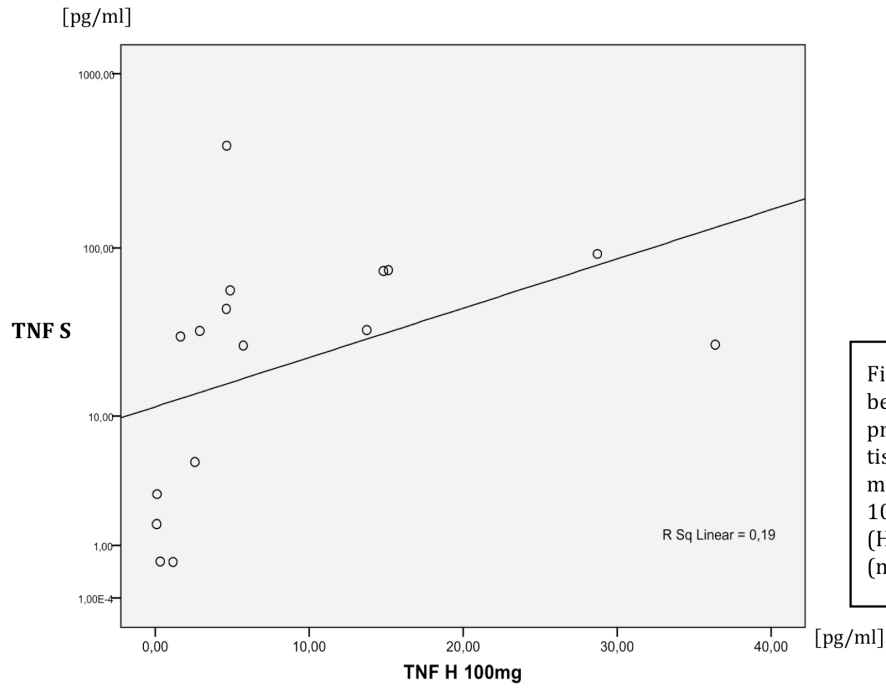


Fig. 12: Correlation between TNF- α -production of 100mg tissue of synovial membrane (S) and 100mg tissue of the IFP (H). (n=16;p<0.01).

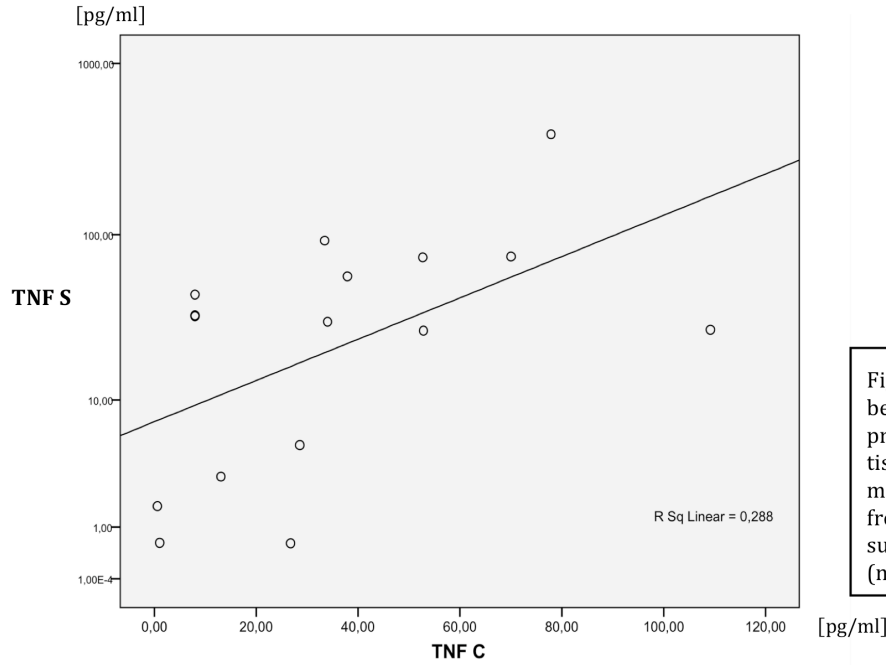
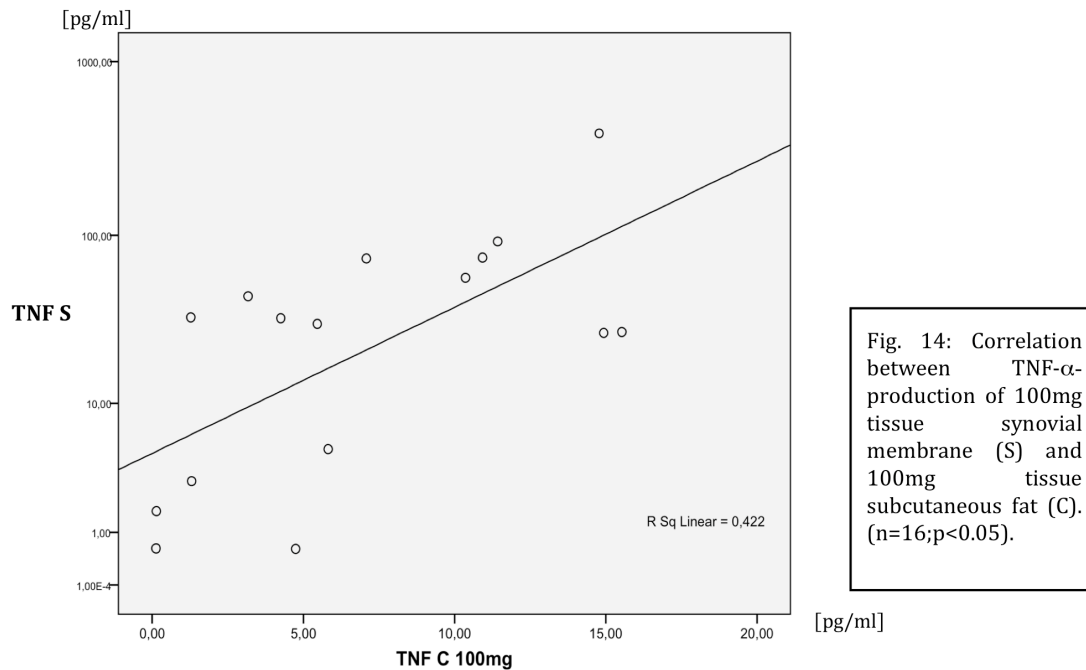


Fig. 13: Correlation between TNF- α -production of 100mg tissue of the synovial membrane (S) and from 1 mio. cells of subcutaneous fat (C). (n=16;p<0.05)



4.3.4 MCP-1

The amount of MCP-1 released from 1 mio. adipocytes of H lay between 40.1 pg/ml and 968.2 pg/ml. The median value was 232.4 pg/ml. The amount of MCP-1 released from 1 mio. cells of C lay between 142.9 pg/ml and 2,711 pg/ml. The median value was 748.9 pg/ml (Figure 15). There were no statistically significant gender-specific differences in the MCP-1 release from H and C. Furthermore, no correlations could be found between MCP-1-release and BMI, age, IFP-weight or adipocyte-volume.

One hundred mg tissue of the IFP (H) produced between 14.5 pg/ml and 408.6 pg/ml MCP-1 and the median value was 83.9 pg/ml. The MCP-1-release from C lay between 15.9 pg/ml and 740.8 pg/ml and the median value was 190.9 pg/ml. One hundred mg of synovia produced between 1,008 pg/ml and 4,901 pg/ml MCP-1 and the median value was 1,462 pg/ml (Figure 16). No statistically significant correlations between BMI, age, IFP-weight, adipocyte-volume and MCP-1 release of H, C or S were observed.

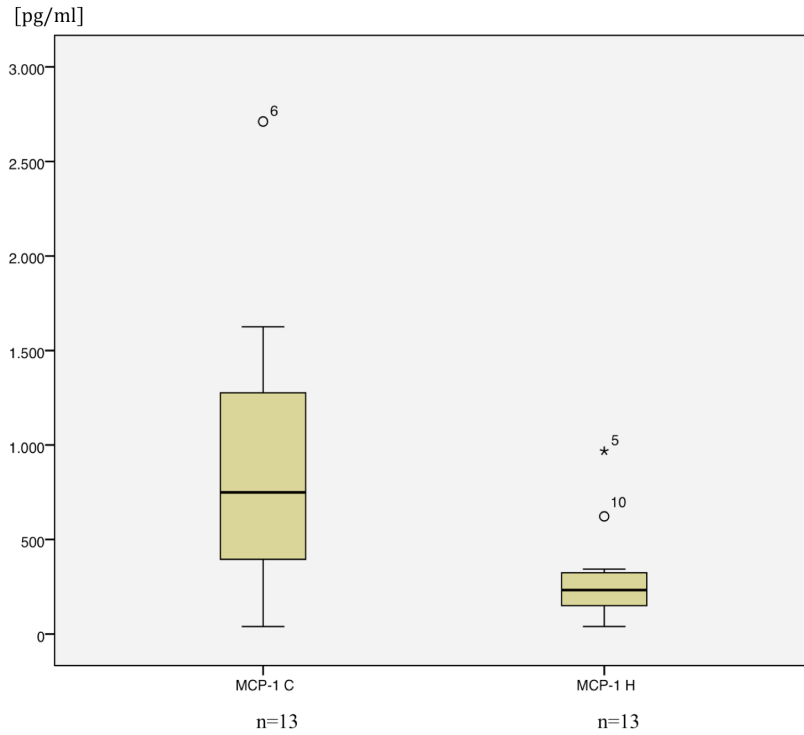


Fig. 15: MCP-1-release from 1 mio. adipocytes of subcutaneous fat (C) and 1 mio. cells of the IFP (H). Results are given as mean values \pm SEM. (p=n.s.)

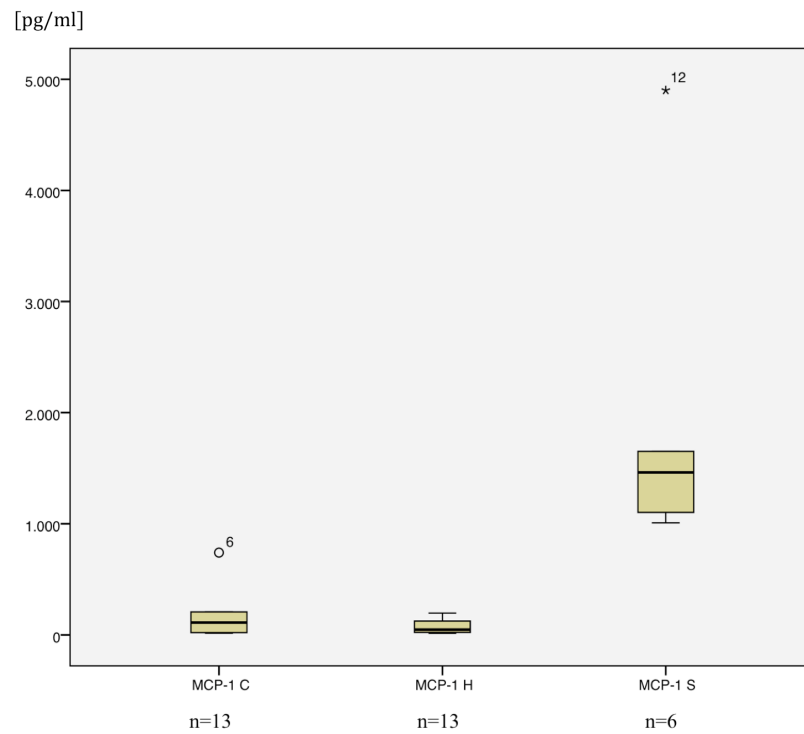


Fig. 16: MCP-1-release from 100mg tissue subcutaneous fat (C), the IFP (H) and synovial membrane (S). Results are given as mean values \pm SEM (p=n.s.)

4.3.5 Adiponectin

The adiponectin-release from 1 mio. cells of H was between 0.9 pg/ml and 14.6 pg/ml and the median value was 3.1 pg/ml. Adipocytes from C produced between 3.1 pg/ml and 35.2 pg/ml adiponectin. The median value was 11.8 pg/ml (Figure 17). No statistically significant gender-specific differences or correlations between BMI, age and adiponectin-release of H or C were found. However, there was a significant correlation between the adipocyte-volume of H and the amount of adiponectin released by H ($r=0.53$; $p<0.05$) (Figure 19). Additionally, the amount of adiponectin released from C correlated with the total weight of the IFP ($r=0.593$; $p<0.05$) (Figure 20).

One hundred mg tissue of the IFP (H) produced between 0.5 pg/ml and 4.7 pg/ml adiponectin and the median value was 1.4 pg/ml. The adiponectin release from C lay between 1.1 pg/ml and 12.2 pg/ml and the median value was 2.7 pg/ml. One hundred mg of synovia produced between 23.1 pg/ml and 85.5 pg/ml adiponectin and the median value was 45.2 pg/ml (Figure 18). No statistically significant gender-specific differences or correlations between BMI, Age, IFP-Weight, adipocyte-volume and adiponectin release of H, C or S were found.

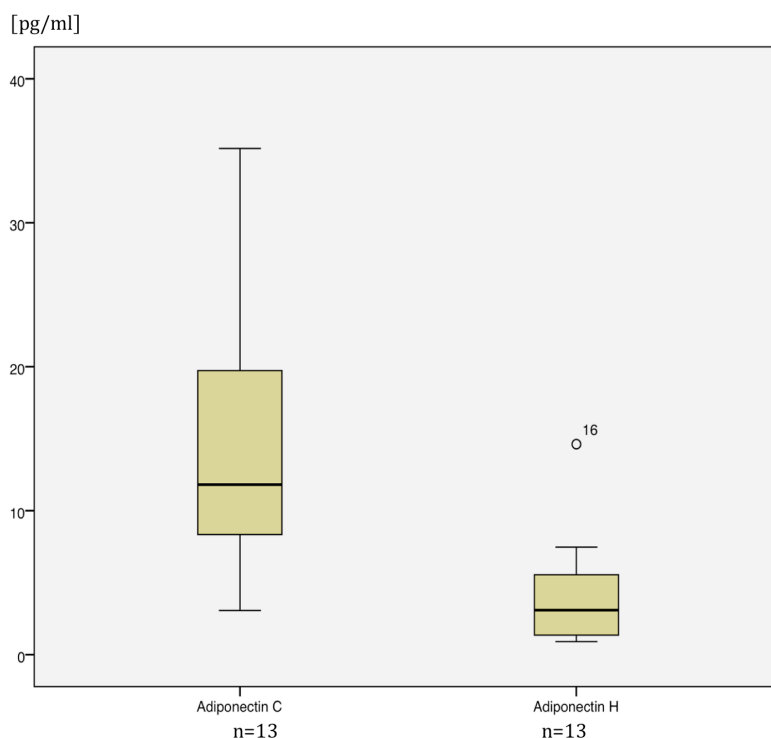


Fig. 17: Adiponectin-release from 1 mio. adipocytes of subcutaneous fat (C) and of 1 mio. cells of the IFP (H). Results are given as mean values \pm SEM ($p=n.s.$).

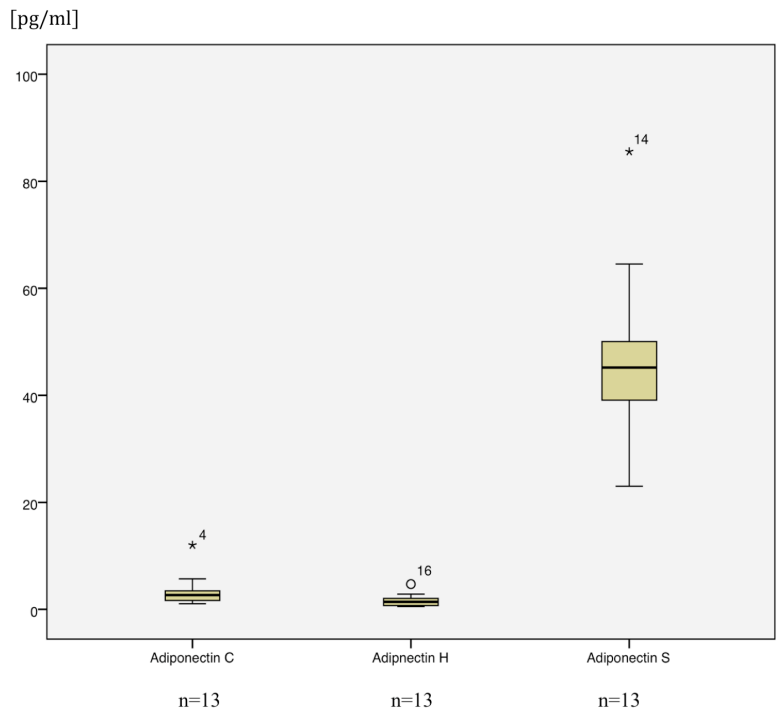


Fig. 18: adiponectin-release from 100mg tissue of the IFP (H), subcutaneous fat (C) and synovial membrane (S). Results are given as mean values \pm SEM (p=n.s.).

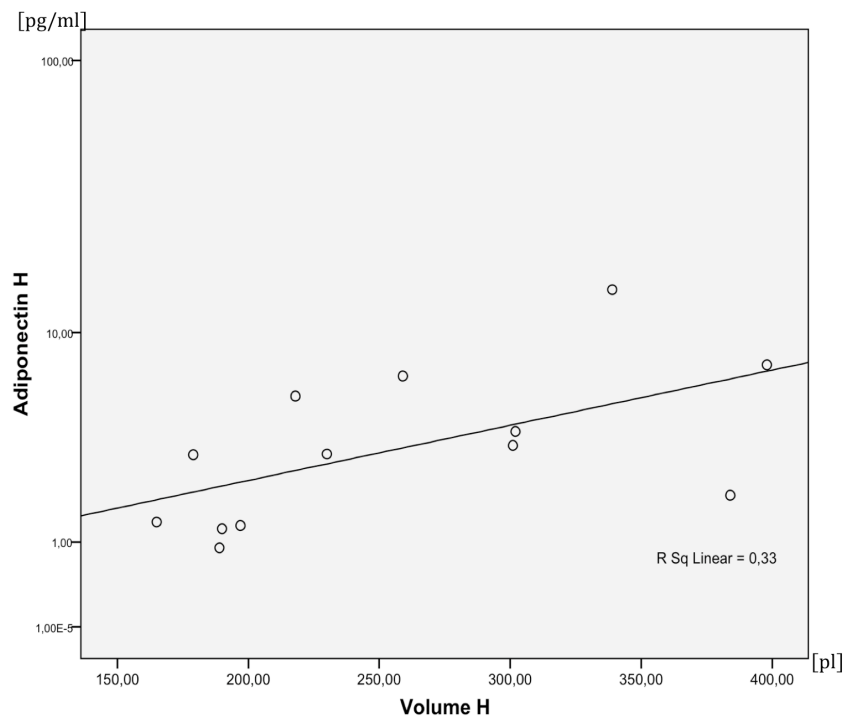
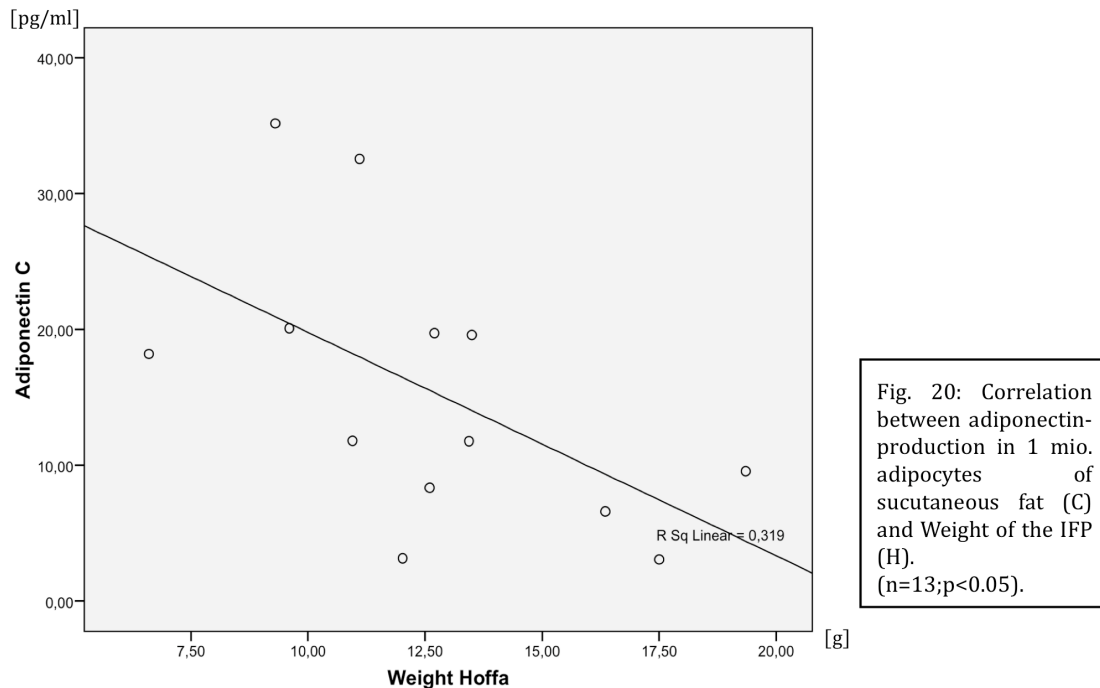


Fig. 19: Correlation between adiponectin-production in 1 mio. cells of the IFP (H) and the Volume of the IFP (H) (n=13) (p<0.05).



4.3.6. Leptin

The Leptin-release from 1 million cells of H was between 0.9 pg/ml and 42.7 pg/ml and the median value was 1.4 pg/ml. C produced between 0.7 pg/ml and 1,632.3 pg/ml Leptin. The median value was 189.1 pg/ml (Figure 21). No statistically significant gender-specific differences or correlations between BMI, age, IFP-weight and leptin release of H or C were found. However, there was a significant correlation between the adipocyte volume of H and the amount of leptin released by H ($r=0.53$; $p<0.01$) (Figure 23), as well as between adipocyte volume of C and the amount of leptin produced by C ($r=0,7$; $p<0.01$) (Figure 25).

The leptin-release from 100 mg of C, H and S showed the following. Levels secreted by H were between 0.5 pg/ml and 18 pg/ml and the median value was 0.7 pg/ml. C produced between 0.4 pg/ml and 284.9 pg/ml and the median value was 40.2 pg/ml. The leptin-release from S lay between 0.9 pg/ml and 169.1 pg/ml and the median value was 51.9 pg/ml (Figure 22). There were no statistical significant gender-specific differences and no correlations between age, IFP-weight, adipocyte-volume and leptin-release from 100 mg tissue of H or C to be found. However, there was a mild correlation between BMI and the leptin-production from H ($r=0.2$; $p<0.05$) (Figure 24). Moreover, the leptin-release of S correlated the adipocyte volume of C ($r=0.54$; $p<0.01$) (Figure 26). The leptin production of S correlated furthermore

with the leptin production of 1 mio. cells of C ($r=0.48$; $p<0.01$) (Figure 27) (as well as with the leptin production of 100 mg tissue of C ($r=0.48$; $p<0.01$) (Figure 28).

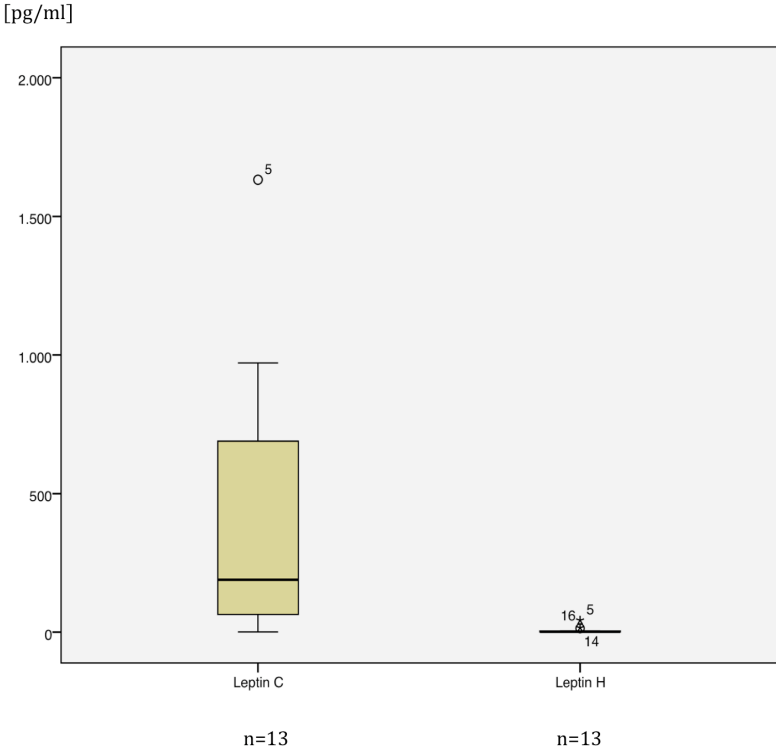
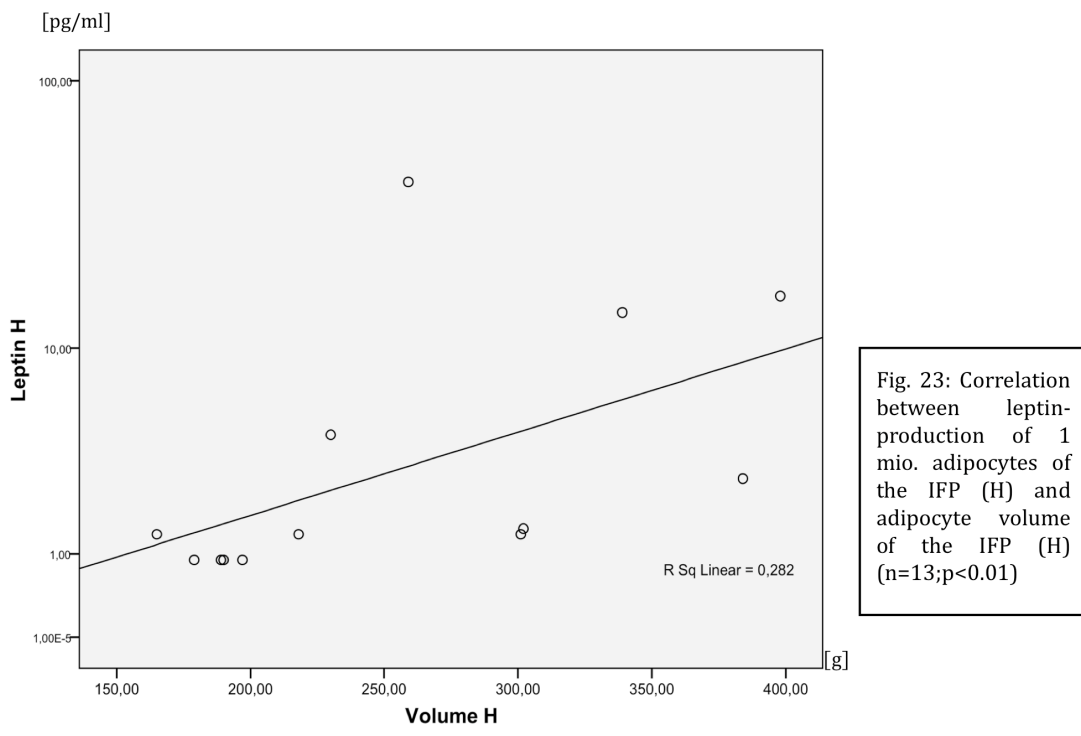
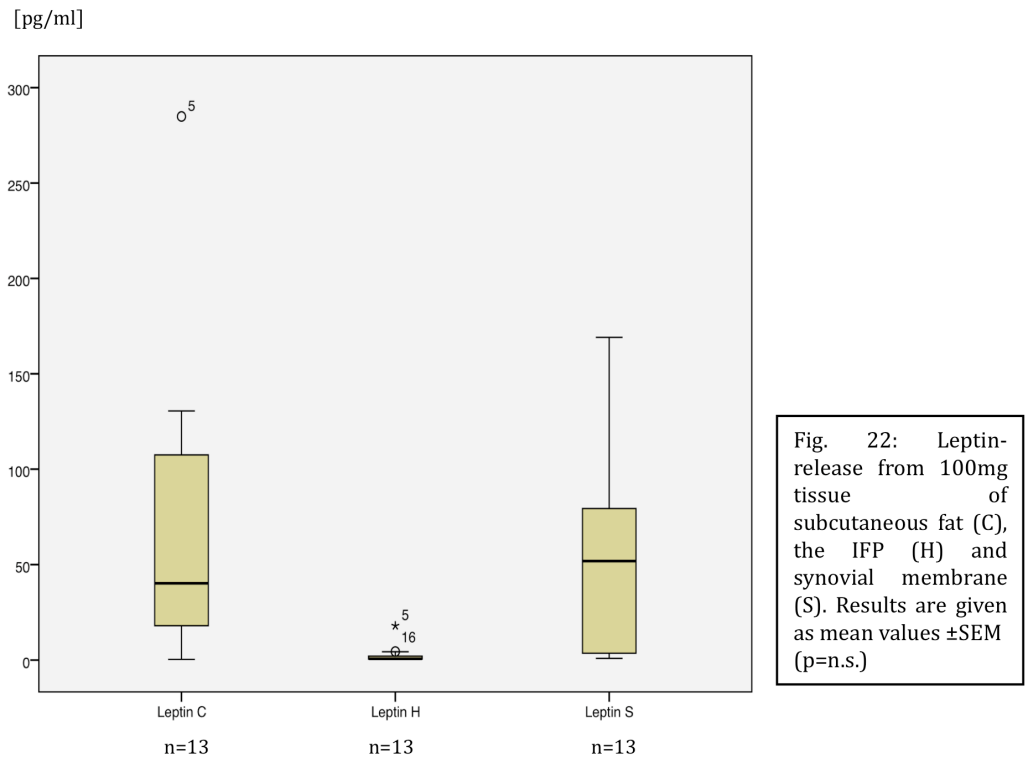


Fig. 21: Leptin-release from 1 mio. adipocytes of subcutaneous fat (C) and from 1 mio. cells of the IFP (H). Results are given as mean values \pm SEM ($p=n.s.$)



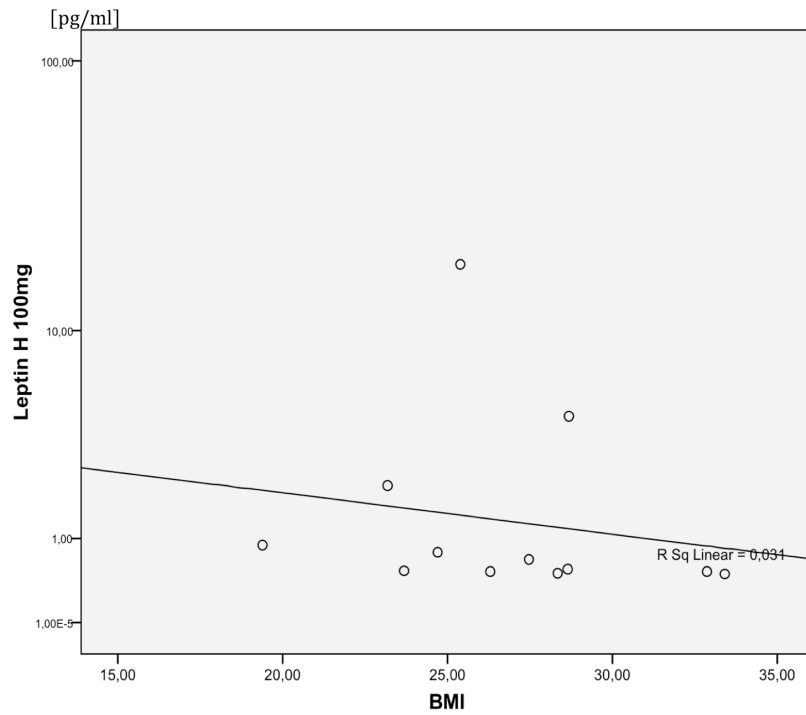


Fig. 24: Correlation between leptin-production in 100mg tissue of the IFP (H) and the body mass index (BMI) (n=13;p<0.05).

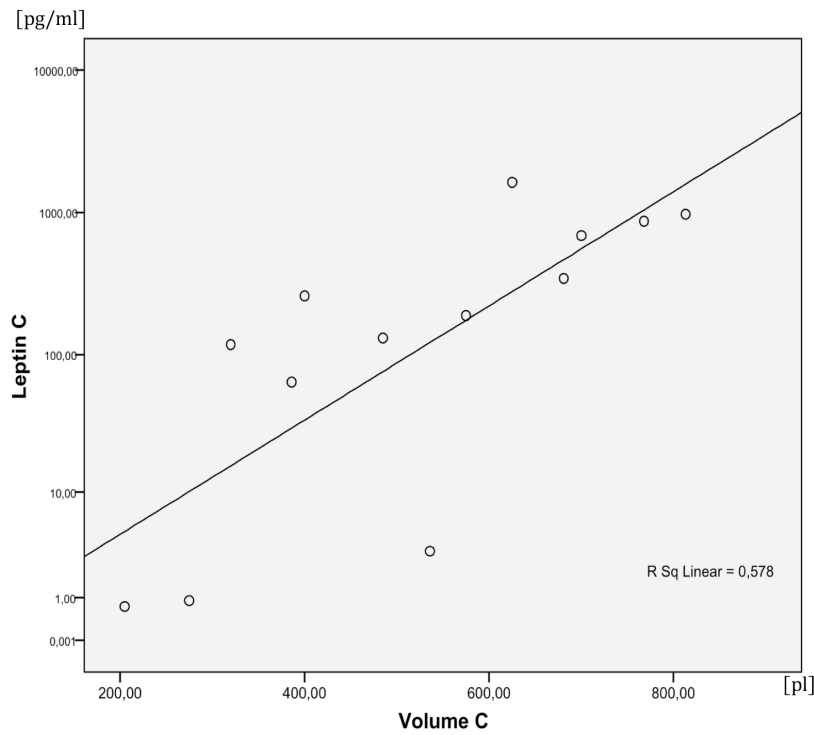


Fig. 25: Correlation between leptin-production in 1 mio. cells of subcutaneous fat (C) and the adipocyte volume of subcutaneous fat (C) (n=13;p<0.01).

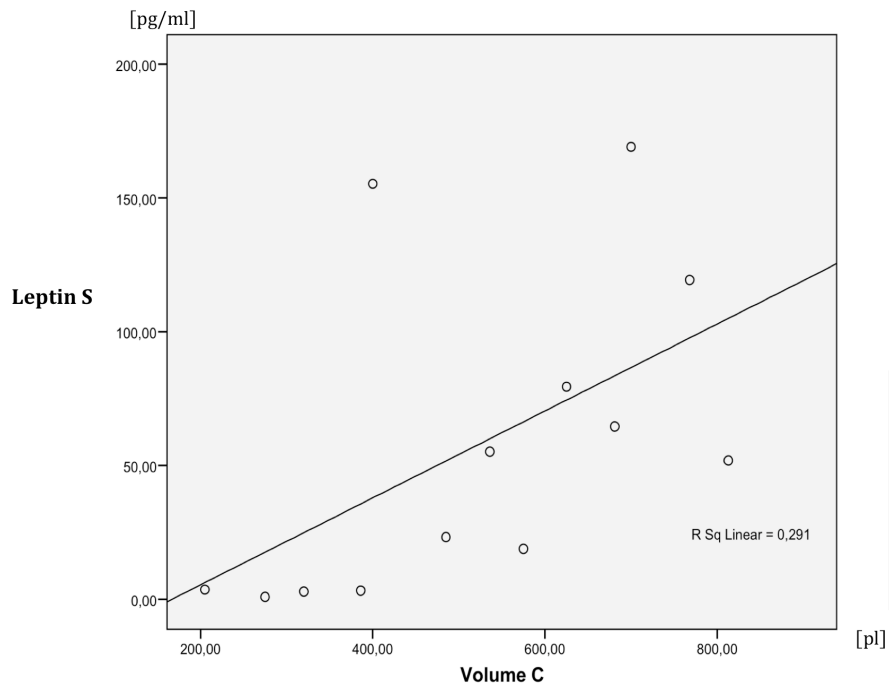


Fig. 26: Correlation between leptin-release of synovial membrane (S) and the adipocyte volume of subcutaneous fat (C) (n=13;p<0.01)

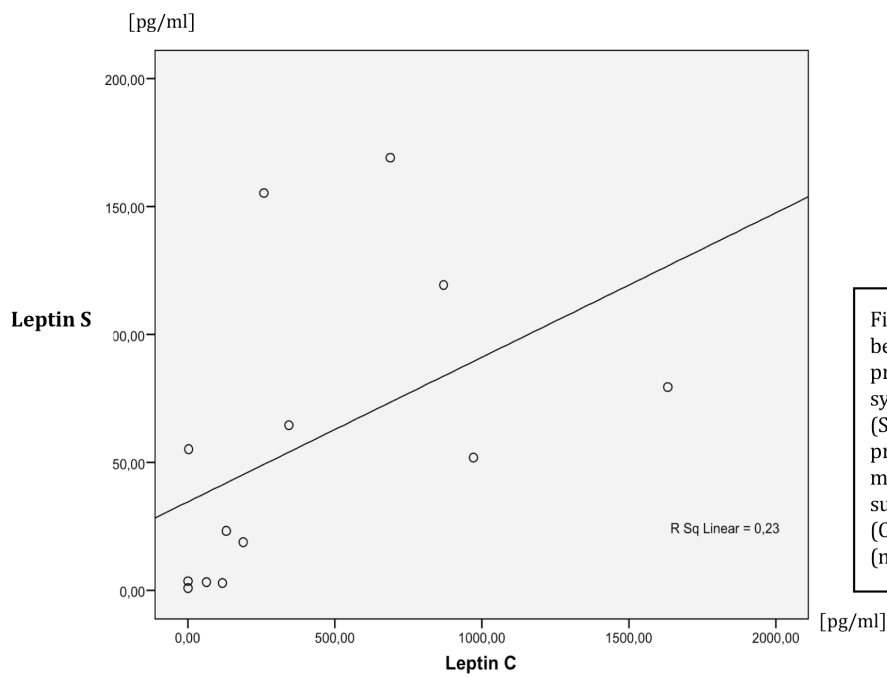
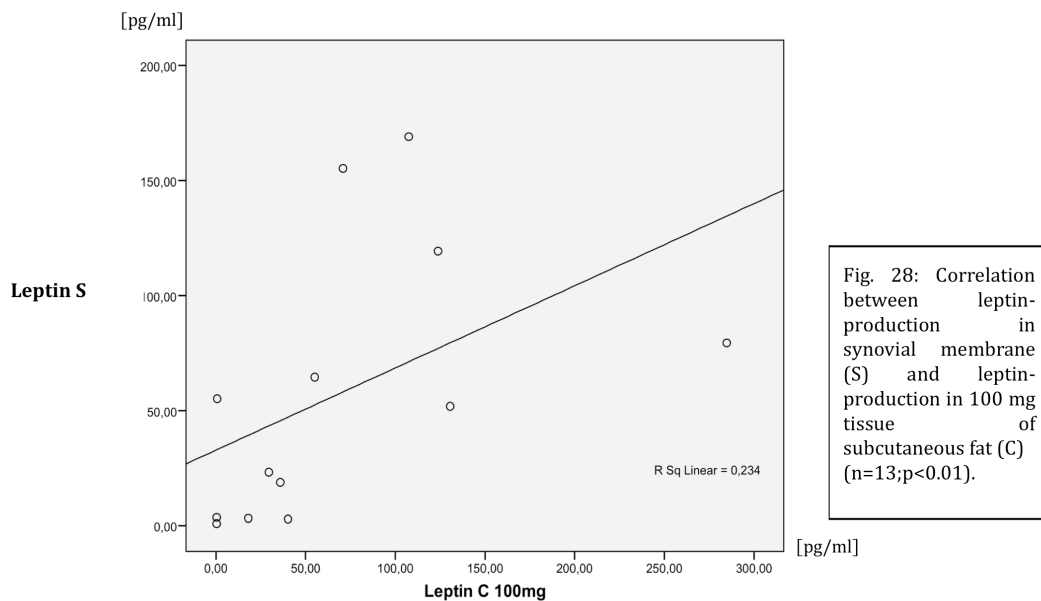


Fig. 27: Correlation between leptin-production synovial membrane (S) and leptin-production in 1 mio. cells of subcutaneous fat (C) (n=13;p<0.01)



4.4 Adipokines found in synovial fluid

Synovial fluid (SYN) is the secretory product from synoviocytes from the inner membrane of the joint capsula. Adipose tissue has a close association with the synovial membrane although no direct connection into the joint space exists. Nevertheless, we wanted to measure the presence of classical adipokines like adiponectin and leptin to show that adipose secreted products are actively released into the joint space. In addition we also wanted to test the hypothesis that other pro-inflammatory secretory products from adipocytes potentially pass over into the synovial fluid.

The results varied greatly but the median value for IL-6, IL-8, TNF- α , MCP-1, Adiponectin and Leptin were 4,406 pg/ml (results lay between 226.6 pg/ml and 27,840 pg/ml), 384.6 pg/ml (btw. 3.96 and 25,385 pg/ml), 8 pg/ml (btw. 0.06 and 34.7 pg/ml), 541.4 pg/ml (btw. 179 and 1,556 pg/ml), 0.9 pg/ml (all samples below detection limit) and 815.2 pg/ml (btw. 42.1 and 3,359 pg/ml), respectively (Figure 29). There were no gender-specific differences and no correlations between BMI, age and IFP-weight could be found. However, there was a statistically significant correlation between leptin in the synovial fluid and adipocyte volume of C ($r=0.71$; $p<0.01$) (Figure 30).

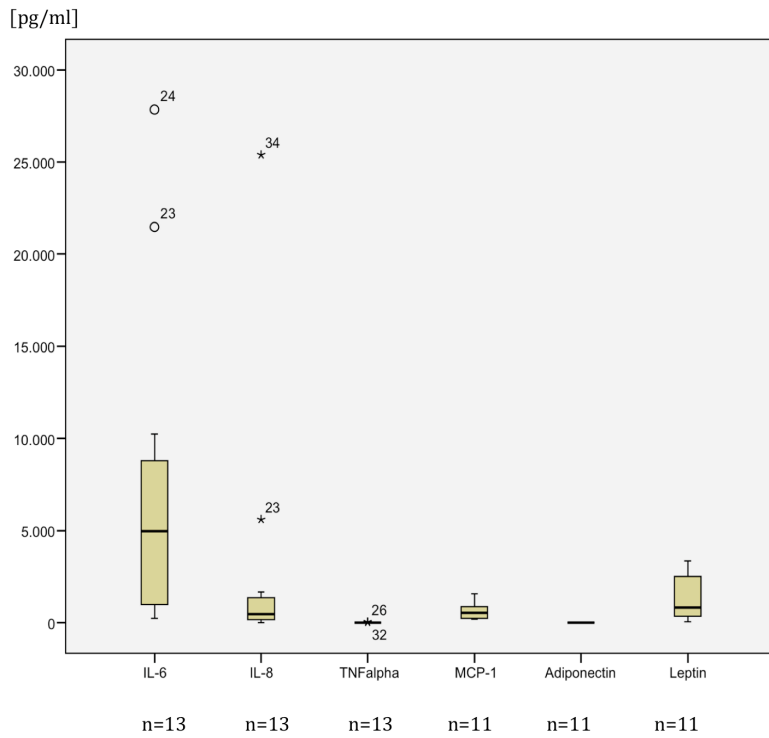


Fig. 29: Amount of IL-6, IL-8, TNF- α , MCP-1, adiponectin and leptin found in Synovial Fluid (SYN). Results are given as mean values \pm SEM (p=n.s.)

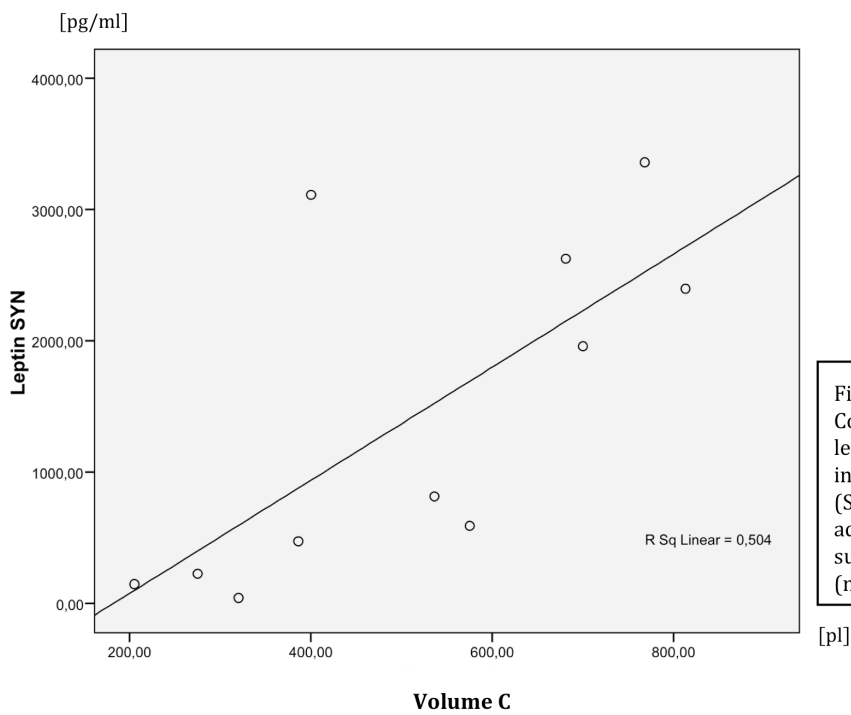


Fig. 30: Correlation between leptin-amount found in Synovial Fluid (SYN) and the adipocyte volume of subcutaneous fat (C) (n=11;p<0.01).

5. Discussion

5.1 Osteoarthritis and cytokines

OA can be seen as a result of destructive cartilage erosion and synovial inflammation. This inflammation is triggered by pro-inflammatory cytokines like IL-1 and TNF- α . Currently it is poorly understood which compartments of the affected joint contribute to the local levels of these pro-inflammatory mediators. Infiltrating immune cells as well as synoviocytes are capable for their production. Moreover, the potency to produce factors which are able to confine the inflammatory process like the IL-1 receptor antagonist (IL-1RA) are reduced (60,122,85). Various strategies like the use of recombinant IL-1RA or neutralizing TNF- α antibodies were used in animal models to minimize cartilage destruction (38). Another optional therapeutic strategy is to use anti-inflammatory cytokines such as IL-10 (122,85).

Nevertheless, as mentioned before, different compartments of diseased joints could contribute to the cytokine release during the initiation and progression of OA. It is now widely accepted that adipose tissue is a secretory organ and has a great potential to release a variety of biologically active compounds. Its activity for inflammatory conditions has to be emphasized in more detail.

5.1.1 Adipose tissue and inflammation

Adipose tissue is an actively secreting endocrine organ (49,116,76,108,109,1,107,89,23,50). It releases amongst others, factors of the complement system, coagulatory proteins, hormones for the regulation of blood pressure and electrolyte homeostasis, but also immune-modulatory factors. Several lines of evidence suggest that obesity is a low-grade systemic inflammatory condition accompanied by increased circulating levels of various cytokines (50,49,116,109). Moreover, several studies were able to provide proof that these cytokines are actually produced in adipose tissue and are therefore called adipokines. Importantly, the circulating levels of such adipokines seem to correlate closely with parameters such as BMI (62,73,17), waist circumference (62) and also importantly, with adipocyte size (94). These inflammatory adipocytokines are considered to play an important role in the development of metabolic and cardiovascular complications in obesity. Regarding OA, it was of great interest for us to

investigate whether adipose tissue of the knee might contribute to this inflammatory, destructive process. We wanted to find out whether adiposity might be a cause of OA not only by adding more weight and therefore more stress to the joint, but also by means of producing pro-inflammatory parameters and actively releasing these into the joint space. A study by Thumb provided proof that certain cytokines (IL-6, TNF- α) are able to mediate cartilage damage (105). Therefore, an increased release of pro-inflammatory cytokines from adipose tissue into the joint could have detrimental effects for the joint cartilage.

Several studies seem to confirm the association between adipose tissue and inflammatory cytokines; such as MCP-1, IL-8, IL-6 or TNF- α . (62,16,17,77). But, there seems to be a discrepancy between cytokine production and the release into the circulation. For example, a study by Mohamed-Ali et al. showed that although both, IL-6 and TNF- α were expressed in subcutaneous adipose tissue, only IL-6 was released into the circulation whereas TNF- α was not and exerts only local effects. In contrast, IL-6 was released from the depot and was calculated to contribute to systemic levels up to 30% (77).

There are several studies showing an accumulation of macrophages into adipose tissue which exhibit a huge potential to secrete inflammatory mediators (114,56). Therefore it seems likely that macrophages are primarily responsible for a majority of adipose tissue derived expression of pro-inflammatory markers, at least for TNF- α . Within this study we collected also tissue specimen for immunohistochemistry and further work has to investigate immune infiltration into the Hoffa fat pad.

As mentioned before, the number of macrophages in adipose tissue is increased in obesity. These cells may also participate in inflammatory reactions. Adipocyte size seems to be a strong predictor of macrophage infiltration into adipose tissue. Moreover adipocyte size increases with obesity. (94) Therefore, with increasing adiposity, adipose tissue may release signals such as MCP-1, causing increased monocyte accumulation. (114,56,34,94).

In this study, it was interesting to find that fat cells from the IFP were much smaller than fat cells from the thigh. Moreover, the adipocyte volume of neither the IFP-cells nor the WAT-cells from the thigh did increase with the BMI. This stands in contradiction to previous studies and seems to indicate that the fat cells from the IFP are not stimulated in a similar fashion as other fat cells in the body.

In conclusion, there is evidence for the systemic effects of adipokines in obesity. The main idea of this study was to investigate a potential association between the secretory properties of fat cells from the Hoffa fat pad and local effects of adipokines in the joint, for presently much less research has been dedicated to this topic. When considering the systemic effects of adipose tissue, it seems plausible to suspect similar effects of a fat pad within the knee in an inflammatory disease such as OA.

5.2 Relation of the fat cell size between Hoffa and control fat and their correlation with clinical parameters

A matter of interest for us was to investigate whether there are significant differences between adipose tissue from the IFP and subcutaneous adipose tissue. It has been shown that visceral, subcutaneous and omental fat are different in various aspects, but so far not much research on the infrapatellar fat has been conducted.

Therefore, we correlated the fat cell volumes with the BMI of the donors, the total weight of the fat pad, age and the secretory activity for IL-6, IL-8, TNF- α , MCP-1, adiponectin and leptin release. On average, adipocytes from subcutaneous fat of the thigh had a higher volume than adipocytes from the IFP (614.1 ± 231.2 pl vs. 282.2 ± 113.6 pl). Interestingly, both of them did not correlate with BMI, age or IFP-weight, although significant associations between BMI or age were demonstrated before in subcutaneous abdominal deposits (112). Interestingly there was a significant correlation between the two volumes of Hoffa and control fat, potentially demonstrating a general mechanism that cell size may be individually fixed. The missing association between BMI and adipocyte size may also demonstrate a distinct function of the two fat depots apart from metabolism known for the various abdominal depots. Unfortunately we had no access to abdominal adipocytes to verify our conclusions.

Many study groups tried to prove the hypothesis that adipocyte size alters cell biology in such a way that more and more cytokines are produced and released into the circulation which consequently causes the chronic inflammatory condition in obesity (7,94). As there was no correlation between fat cell size and BMI we can not draw any conclusion that a positive association could have been a proof for inflammation in the local context of OA in our study.

It has been shown that TNF- α and IL-6 levels were positively and adiponectin negatively correlated with adipocyte size (7). In a different study, leptin secretion and circulating plasma

leptin levels were directly proportional to adipocyte volume (94). Our results demonstrated, that when calculated per one million cells, the secretion of adiponectin and leptin in the IFP correlated significantly positively with the adipocyte volume of the IFP and leptin production in subcutaneous fat correlated as well with adipocyte volume of this fat sample. When normalized for 100 mg tissue, no correlations between any cytokine secretion and adipocyte volume could be detected. Because the results for 1 Mio. cells are in agreement with the current literature, it seems that this is a more exact way to assess the results than the calculation per 100mg tissue.

5.3 Evidence of adipokine production in the infrapatellar fat-pad

Recently, some studies investigated the cytokine production in the infrapatellar fat pad (87,110). Ushiyama et al (110) showed that infrapatellar fat pad specimens contained bFGF, VEGF and the proinflammatory cytokines TNF- α and IL-6. The infrapatellar fat pad was found to actively synthesize these cytokines. The authors concluded that although synovial cells and articular chondrocytes expressed these cytokines and are thought to be the primary sources of those in the joint cavity, they may also originate from the infrapatellar fat pad. The classical adipokine in this respect is leptin. Its expression is mostly related to fat cells, but interestingly also different structures of the knee joint were also shown to produce this peptide hormone (87). Presle et al were also able to show that osteophytes released larger amounts of leptin than did the synovial membrane or the IFP (87). This may be explained by the same origin of osteoblast and adipoblasts. Repeatedly, it was suggested that leptin had a key function in the course of OA (104, 29). Also we were able to measure leptin in significant quantities in the SYN. In contrast to a recent work showing that leptin concentrations in the SYN correlated with the body mass index (29) we were not able to confirm this association. Nevertheless, there were significant associations between leptin concentrations in the SYN and the volume of subcutaneous control fat from the thigh. There are two possibilities to explain this association. First of all, it is possible that leptin may diffuse into the joint space from fat tissue surrounding the knee and secondly, it might be possible that the increasing fat cell size from the WAT might somehow stimulate the synovial membrane to produce leptin and release it into the joint space (the amount of leptin produced in the synovial membrane did indeed correlate with the fat cell size of WAT of the thigh and the amount of leptin produced in those WAT cells).

We also found that an increased adipocyte size of the IFP and the control fat correlated with the amount of leptin produced in each of these cells.

In addition, in our study, we found that fat cells from the IFP produced a variety of other adipokines. Foremost, they were found to release IL-8 and IL-6 and to a minor extent TNF- α , MCP-1, adiponectin and leptin. We also compared the adipokine release of the IFP to the adipokine release of subcutaneous adipose tissue from the thigh, in order to investigate whether adipose tissue of the IFP responds in the same way to stimuli (i.e. obesity) as normal adipose tissue does. Interestingly, adipose tissue of the thigh exceeded the IFP in the production of MCP-1, adiponectin and leptin by far. This could indicate that fat cells from the IFP are not stimulated in the same way as WAT-cells.

We also wondered whether the total weight of the IFP would be affected by the patient's body weight and would therefore increase simultaneously with the BMI, but no such correlation was found. The reason may be that some patients were already re-operated and only residues of the Hoffa fat body were removed in the second operation, which made it difficult in some cases to evaluate the exact margins of the IFP and scar tissue.

5.4 Adipokines in synovial fluid (SYN) and its association to the release from the IFP

As discussed before, even though adipose tissue in fact produces high amounts of adipokines, this does not necessarily implicate, that these adipokines are also released into the circulation or in our case into the synovial fluid. Therefore, we also examined synovial fluid in order to establish a link between adipokine-production in the IFP and an active release into the joint cavity, where the adipokines might possibly contribute to the destruction of articular cartilage. Unfortunately it was not possible to obtain SYN from all patients, as some of them had hardly any fluid in their inflammatorily altered joints, some of them were bloody according to the operating procedure and others were clotted according to the inflammatory process.

It was shown that a healthy joint shows no detectable amounts of proinflammatory cytokines like IL-6, IL-8, or TNF- α (59). To our knowledge, no study so far has demonstrated the presence of leptin, adiponectin and MCP-1 found in an uninjured joint so far. In our study we measured particularly high levels of IL-6 and less high levels of leptin, IL-8 and MCP-1 but it

is not clear whether the adipokines measured were directly released into the joint space by surrounding fat or display an active filtration from the blood stream.

Presle et al (87) analysed the quantity of leptin, resistin and adiponectin in synovial fluid of the knee joint in patients suffering from OA and found elevated levels of leptin in synovial fluid when compared to plasma levels, but only in women. They suggest that circulating levels of adipokines in the body are not necessarily consistent with the amount of adipokines in a joint. Moreover, they analysed the amount of soluble leptin receptor, as this isoform may determine the biological activity of this adipokine. The receptor was detected in synovial fluid obtained from patients with OA, but at a lower level compared to serum values. The high level of leptin associated with a decline in soluble-receptor-level in the joint compartment led to a large rise in bioavailable leptin in synovial fluid. Thus, the presence of high level of bioactive leptin in the joint from OA patients may have pathophysiological implications and could explain the higher incidence of OA in women as a local effect of leptin. In our study, there were tendentially higher amount of leptin in the SYN samples of female patients, but unfortunately these results did not reach statistical significance.

Presle et al. also raised the question whether leptin might be transported into the joint due to an increased permeability of the inflamed synovial membrane. However the level of resistin in synovial fluid was much lower compared to the corresponding value found in the serum. Since both adipokines have a similar molecular weight and should therefore both be transported into the joint to a similar degree, the high level of leptin in the synovial fluid points to a local production in the joint.

In our collective of OA-patients the level of adiponectin was very low in synovial fluid and was hardly detectable in any of the samples. Our findings therefore confirm a recent other study (23). In this particular study by Chen et al. most of the adiponectin found in synovial fluid consisted of the trimer form and not the high molecular weight form (HMW). The authors therefore speculated, that the the smaller, trimer form might pass the synovial membrane more easily than the bigger, high molecular from. They concluded that the adiponectin found in synovial fluid must not necessarily be produced in a structure of the knee, but might also pass the inflamed synovial membrane and originate from other structures.

In our study, we found high amounts of IL-6 in the synovial fluid, as well as IL-8 and leptin. The amount of adipokines found in synovial fluid did not correlate with the BMI or the adipocyte size from the IFP. But the leptin-concentration in the SYN did correlate with the

adipocyte size of the subcutaneous fat tissue, raising therefore the question, whether leptin is transported from adipose tissue outside of the joint into the synovial fluid or whether other structures of the knee beside the IFP (i.e. synovial membrane) release leptin into the joint cavity.

5.5 Production of cytokines from other structures in/surrounding the knee (WAT of the thigh and synovial membrane)

As explained above, other structures in the knee (especially synovial membrane) possess the ability to produce adipokines. It is therefore of interest to compare the secretion of such factors from these structures with that from the IFP. The study from Presle et al. (87) demonstrated that the synovial membrane and the IFP released large amounts of leptin. However, osteophytes (bone spurs that form due to increased damage of the joint in OA) were shown to represent the major source of intra-articular leptin production while adiponectin production in osteophytes was similar to other joint tissues (87). Unfortunately, we did not test osteophyte-tissue in our study, but we were able to confirm that the synovial membrane and the IFP indeed both produce leptin. In contrast to the study by Presle et al, our samples of synovial membrane and also samples of the WAT exceeded the IFP by far in leptin-production.

Bondeson et al. (12) examined the secretion of immune mediators of synovitis in OA in more detail. According to them the overproduction of cytokines and growth factors from the inflamed synovial membrane may play a critical role in the pathophysiology of OA. They suspected that in particular TNF- α and IL-1 are the key players in OA pathogenesis, both in synovial inflammation and in the activation of chondrocytes. These cytokines can stimulate their own production and induce IL-6 and IL-8 production in synovial cells and chondrocytes. Bondeson et al. (12) expected synovial macrophages to be responsible for maintaining synovial inflammation in OA, similar to RA, where macrophages are the main promoter of the disease activity. Indeed, synovial macrophage differentiation differed between inflammatory and non-inflammatory OA. Particularly in the early stage of OA, the synovitis showed a mononuclear cell infiltrate, with considerable production of proinflammatory cytokines and destructive enzymes.

Furthermore, another study clearly demonstrated that not only articular adipose tissue but also the synovial membrane of patients with OA is a significant source of adiponectin (32). Although adiponectin has been reported to have a protective rather than a proinflammatory or destructive role, in this study, adiponectin seems to exert significant proinflammatory and matrix-degrading effects (23,32). The authors observed an up-regulation of IL-6 by adiponectin. Other proinflammatory cytokines, such as IL-1, TNF- α and VEGF, as well as protective cytokines such as IL-4 and IL-10 were not affected. On the other hand, a different group reported a protective role of adiponectin in cartilage-degradation (23). These contradicting results may be explained by the fact, that adiponectin acts in a pro- or anti-inflammatory way, depending on its molecular form. It has been shown that the various molecular forms of adiponectin can act on many, very different systems in the human body (79). This should be taken into consideration, because in our study, we only measured the high molecular form of adiponectin.

All in all, it seems that chronic inflammatory changes with the production of proinflammatory cytokines are a key feature of synovial membranes from patients with OA, with the most severe changes seen in patients at the time of joint replacement surgery resembling those seen in RA. This low-grade synovitis results in the production of cytokines that may contribute to the pathogenesis of OA (68,95,85). Our results demonstrate, that the synovial membrane is highly active in producing not only IL-6, IL-8 and TNF- α but also MCP-1, adiponectin and leptin when compared to the IFP. Except for leptin, the synovial membrane also released far more of the before mentioned cytokines than subcutaneous adipose tissue. The production of leptin in the synovial membrane correlated with the patient's BMI and with the adipocyte size of subcutaneous adipose tissue. These results raise the question whether an increase in body weight and therefore an increase of adipocyte volume might somehow trigger the production of leptin in the synovial membrane.

Because cartilage degradation is one of the key features in OA, naturally it became of interest, which cytokines contribute to cartilage damage and whether cartilage itself releases cytokines as an indicator or contributor to the pathologic changes observed in OA. Various studies provide proof that cartilage does express cytokines, but the results are not yet completely clear. For example, Moos et al. detected two different expression patterns in cartilage from OA patients. TNF- α and IL-6 were either very low or very high. The author thus concluded that there are different cytokine-pathways leading to the same destruction of cartilage (78).

When injected into the joints of rats, leptin stimulated expression of two cartilage growth factors, insulin-like growth factor-1 (IGF-1) and transforming growth factor- β (TGF- β), and was accompanied by an increase in proteoglycan synthesis (65). Also, functional leptin receptors were detected on human adult articular chondrocytes, and treatment of isolated cells in vitro was shown to stimulate cell proliferation as well as matrix synthesis. These findings raise the question whether leptin could influence articular cartilage metabolism and furthermore provide a metabolic link between obesity and OA. Leptin concentrations of 10-100 ng/ml stimulate chondrocyte proliferation, while lower concentrations of 0.1–1 ng/ml stimulate proteoglycan and collagen synthesis (40). Whether these levels of leptin might be present in vivo is not known. The concentration of leptin in normal cartilage has not been measured. Unlike the highly positive immunostaining for leptin seen in osteoarthritic cartilage, few cells staining positively for leptin were observed in normal cartilage. The latter finding does not exclude a role for leptin in normal cartilage homeostasis. In the absence of knowledge of the function of leptin in cartilage, it is not clear whether increased production of leptin would be beneficial or detrimental (65).

Other studies speculate about a different role of leptin in OA. In cultured human chondrocytes, leptin increased both the proliferation and the extracellular matrix synthesis, but in a biphasic manner, with a reduced stimulating effect at the highest concentrations. Leptin may thus have a beneficial effect on cartilage synthesis either directly or through the upregulation of growth factors. However, an excess of leptin may account for decreased extracellular matrix synthesis and may lead to lesions similar to those found in OA with a high intra-articular level of growth factors (111,29). The increased expression of leptin in markedly damaged cartilage suggests that leptin may trigger cartilage destruction, especially when associated with some local factors. The adipokine synergises with proinflammatory cytokines, such as interleukin 1, to increase nitric oxide production, which is known to interfere with chondrocytes function resulting in the loss of cartilage matrix through induction of apoptosis, activation of metalloproteinases, and inhibition of proteoglycan and type II collagen synthesis (82).

5.6 Cytokine production in the synovial membrane

As the synovial membrane is known to be a major source for inflammatory cytokines, we were interested to find out, whether the inflammatory reaction in the synovial membrane is possibly stimulated by adipose tissue in a paracrine fashion. Therefore, we correlated adipokine production in the synovial membrane with the subcutaneous fat and the

infrapatellar fat pad. Clearly, in our results, the synovial membrane produced exceedingly more amounts of adipokines than the adipose tissues in question. The amount of leptin produced in the synovial membrane correlated with the amount produced in subcutaneous fat tissue. The amount of TNF- α even correlated with both, the subcutaneous adipose fat and the infrapatellar fat. We can therefore speculate, that particularly in the case of TNF- α , there might exist an interaction between the synovial membrane and the surrounding fat tissues.

5.7 Osteoarthritis- a systemic disease?

Finally, one of our main interests was to learn more about the underlying mechanisms that drive OA. Obesity predisposes to OA, with an estimated 9–13% increased risk for the onset of the disease at the knee and also at the hand with every kilogram increase in body mass. Similarly, for every 5 kg gain in mass, the risk of developing knee OA increases by 35% (103).

Furthermore, Toda et al showed that a change in body fat, but not body weight was related to symptomatic relief of obese patients with knee OA (106).

Although it is still controversial whether OA can be considered a systemic disease (102,103,52), the results from above clearly contradict the hypothesis that obesity predisposes to OA merely by increasing stress on weight-bearing joints (86,65,80,5,103,43,48).

Our results and other studies provide proof, that various structures in the knee joint produce adipokines. Furthermore, it was possible to detect these adipokines in synovial fluid. However, it is unclear whether the adipokines found in the synovial fluid of OA joints are predominantly a local phenomenon or whether they are a result of systemic causes, such as for example the diffusion of adipose-derived leptin across the synovial membrane (103).

We can find further proof of the damaging effects of cytokines in the knee through several studies evaluating the likelihood of OA following a tear of the anterior cruciate ligament. (20,19). Approximately 44% of patients develop OA following rupture of the anterior cruciate ligament (ACL) if the injury is left unrepaired. Restoring knee stability through reconstruction, while providing symptomatic relief, has not been shown to reduce the incidence of degenerative changes. In fact, recent studies have shown that 50%-60% of ACL-reconstructed patients go on to develop degenerative changes or frank OA. Therefore, the cause of post-traumatic OA might be not biomechanical but biochemical. To test this hypothesis, levels of cytokines which are important in modulating physiological and pathophysiological

metabolism of cartilage in knee joint synovial fluid following ACL rupture were measured. Of great interest was the fact that the levels of these cytokines were very similar in patients 4 weeks after injury and in chronic patients, leading to the hypothesis that a chronic smoldering inflammatory reaction persists after resolution of the acute effusion.

In conclusion, we were able to demonstrate that the infrapatellar fat pad, the synovial membrane and also subcutaneous fat near the knee joint are all secretory active tissues and might therefore contribute to the pathophysiological changes seen in OA. Further research should be done to evaluate the exact importance of this dysregulation within structures of the knee in causing or driving OA.

6. Summary

Several studies have demonstrated that fat tissue is much more than merely a place of storage for energy- it is also a secretory highly active organ. The production and release of so-called “adipokines” can result in a chronic low-grade systemic inflammation which contributes to the increased incidence of diseases such as atherosclerosis or diabetes in the obese population. So far, the infrapatellar fat pad (Hoffa fat pad) has drawn very little attention. Most studies focused mainly on the so-called “Hoffa disease”, a rare condition which is said to lead to chronic knee pain.

As the interest in fat tissue was heightened, it became clear, that there are several different kinds of fat which respond differently to various stimuli and that fat tissue itself consists of several different types of cells, which might influence an inflammatory disease in many ways.

It was the aim of our study to examine the infrapatellar fat pad (IFP) further; whether its adipocytes differ from other adipocytes and whether there is a link between inflammatory adipokines produced in the fat pad and the occurrence and progress of the inflammatory disease “osteoarthritis”.

We therefore collected samples of patients who suffered from advanced osteoarthritis and were scheduled for total knee arthroplasty. Specimens of the infrapatellar fat pad, white adipose tissue (control fat) from the thigh, synovial membrane and synovial fluid from 37 patients were collected. We isolated the adipocytes and measured the following adipokines by means of immunoassays: IL-6, IL-8, TNF- α , MCP-1, Adiponectin and Leptin. These parameters were correlated with the following clinical parameters: Age, Gender, BMI, Weight of the infrapatellar fat pad and the volume of adipocytes from the IFP and from white adipose tissue (WAT) from the thigh.

Briefly, our results were the following. The synovial membrane produced by far the highest amount of adipokines, followed by control fat from the thigh and the infrapatellar fat pad produced adipokines to a lesser extent than the other specimens. Importantly, we found that the volume of adipocytes from the infrapatellar fat pad did increase with the volume of fat cells from the thigh but unfortunately, there was no correlation between fat cell size and the BMI, which according to the current literature could have been expected. Moreover did the adiponectin-productin of the infrapatellar fat pad correlate with the volume of these cells. The amount of leptin produced in adipocytes from the IFP and from WAT correlated beautifully

with the increasing volume of each of these cells. Interestingly, the amount of leptin found in synovial fluid correlated with the volume of WAT-cells from the thigh. This raised the question whether leptin might diffuse from structures further away from the knee into the joint space or whether the synovial membrane might be stimulated to produce increased amounts of leptin. The latter argument is further supported by the fact that the amount of leptin from the synovial membrane correlated with the size of WAT-cells.

All in all, we were able to prove that the infrapatellar fat pad is indeed a secretory active organ, but not the most prominent producer of the investigated specimens. Control fat from the thigh and the synovial membrane exceeded the Hoffa fat pad in adipokine-secretion. We can not say for sure which mechanisms affect the Hoffa fat cells, but it seems that there are other factors involved than in WAT- or visceral fat-cells. Moreover, the fact that we found adipokines not only in the tissue samples but also in synovial fluid indicates that osteoarthritis might indeed be also a systemic inflammatory disease and not just a condition caused by mechanical irregularities.

7. Literature

- (1) Ahima RS. Adipose tissue as an endocrine organ. *Obesity* (Silver Spring). 2006 Aug;14 Suppl 5:242S-249S
- (2) Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. *Adv Immunol.* 1993;54:1-78.
- (3) Ambrosini G, Nath AK, Sierra-Honigmann MR, Flores-Riveros J. Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1. *J Biol Chem.* 2002 Sep 13;277(37):34601 – 34609. Epub 2002 Jun 25.
- (4) Anderson JJ, Felson DT. Factors associated with osteoarthritis of the knee in the first national Health and Nutrition Examination Survey (HANES I). Evidence for an association with overweight, race, and physical demands of work. *Am J Epidemiol.* 1988 Jul;128(1):179–189.
- (5) Aspden RM, Scheven BA, Hutchison JD. Osteoarthritis as a systemic disorder including stromal cell differentiation and lipid metabolism. *Lancet.* 2001 Apr 7;357(9262):1118-20.
- (6) Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett.* 1992 Jul 27;307(1):97-101.
- (7) Bahceci M, Gokalp D, Bahceci S, Tuzcu A, Atmaca S, Arikan S. The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? *J Endocrinol Invest.* 2007 Mar;30(3):210-4.
- (8) Bastard JP, Maachi M, Van Nhieu JT, Jardel C, Bruckert E, Grimaldi A, Robert JJ, Capeau J, Hainque B. Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. *J Clin Endocrinol Metab.* 2002 May;87(5):2084-9.
- (9) Berner HS, Lyngstadaas SP, Spahr A, Monjo M, Thommesen L, Drevon CA, Syversen U, Reseland JE. Adiponectin and its receptors are expressed in bone-forming cells. *Bone.* 2004 Oct;35(4): 842–849.
- (10) Bohnsack M, Meier F, Walter GF, Hurschler C, Schmolke S, Wirth CJ, Rühmann O. Distribution of substance-P nerves inside the infrapatellar fat pad and the adjacent synovial tissue: a neurohistological approach to anterior knee pain syndrome. *Arch Orthop Trauma Surg.* 2005 Nov;125(9):592-7.
- (11) Bokarewa M, Bokarew D, Hultgren O, Tarkowski A. Leptin consumption in the inflamed joints of patients with rheumatoid arthritis. *Ann Rheum Dis.* 2003 Oct;62(10);:952–956.
- (12) Bondeson J, Wainwright SD, Lauder S, Amos N, Hughes CE. The role of synovial macrophages and macrophage-produced cytokines in driving aggrecanases, matrix metalloproteinases, and other destructive and inflammatory responses in osteoarthritis. *Arthritis Res Ther.* 2006;8(6):R187.
- (13) Bornstein SR, Licinio J, Tauchnitz R, Engelmann L, Negrão AB, Gold P, Chrousos GP. Plasma leptin levels are increased in survivors of acute sepsis: Associated loss of diurnal rhythm, in cortisol and leptin secretion. *J Clin Endocrinol Metab* 1998 Jan;83(1):280-283.
- (14) Bruun JM, Lihn AS, Madan AK, Pedersen SB, Schiøtt KM, Fain JN, Richelsen B. Higher production of IL-8 in visceral vs. subcutaneous adipose tissue. Implication of

nonadipose cells in adipose tissue. *Am J Physiol Endocrinol Metab.* 2004 Jan;286(1):E8-13. Epub 2003 Sep 16.

(15) Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab.* 2003 Sep;285(3):E527-33. Epub 2003 May 7.

(16) Bruun JM, Pedersen SB, Kristensen K, Richelsen B. Opposite regulation of interleukin-8 and tumor necrosis factor-alpha by weight loss. *Obes Res.* 2002 Jun;10(6):499-506.

(17) Bruun JM, Pedersen SB, Richelsen B. Regulation of interleukin-8 production and gene expression in human adipose tissue in vitro. *J Clin Endocrinol Metab.* 2001 Mar;86(3):1267-73.

(18) Butler WJ, Hawthorne VM, Mikkelsen WM, Carman WJ, Bouthillier DL, Lamphiear DE, Kazi IU. Prevalence of radiologically defined osteoarthritis in the finger and wrist joints of adult residents of Tecumseh, Michigan, 1962-65. *J Clin Epidemiol* 1988, 41(5):467-473.

(19) Cameron M, Buchgraber A, Passler H, Vogt M, Thonar E, Fu F, Evans CH. The natural history of the anterior cruciate ligament-deficient knee. Changes in synovial fluid cytokine and keratan sulfate concentrations. *Am J Sports Med.* 1997 Nov-Dec;25(6):751-4.

(20) Cameron ML, Fu FH, Paessler HH, Schneider M, Evans CH. Synovial fluid cytokine concentrations as possible prognostic indicators in the ACL-deficient knee. *Knee Surg Sports Traumatol Arthrosc.* 1994;2(1):38-44.

(21) Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest.* 2003 May;111(9):1409-1421

(22) Charriere G, Cousin B, Arnaud E, André M, Bacou F, Penicaud L, Casteilla L. Preadipocyte conversion to macrophage. Evidence of plasticity. *J Biol Chem.* 2003 Mar 14; 278(11): 9850-9855.

(23) Chen TH, Chen L, Hsieh MS, Chang CP, Chou DT, Tsai SH. Evidence for a protective role for adiponectin in osteoarthritis. *Biochim Biophys Acta.* 2006 Aug;1762(8):711-8. Epub 2006 Jul 1.

(24) Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 1996 Feb 1;334(5):292-5.

(25) Cousin B, Munoz O, Andre M, Fontanilles AM, Dani C, Cousin JL, Laharrague P, Casteilla L, Pénicaud L. A role for preadipocytes as macrophage-like cells. *FASEB J.* 1999 Feb;13(2): 305-312.

(26) Curat CA, Miranville A, Sengenès C, Diehl M, Tonus C, Busse R, Bouloumié A. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. *Diabetes.* 2004 May;53(5):1285-92.

(27) Dahlmann I, Kaaman M, Olsson T, Tan GD, Bickerton AS, Wåhlén K, Andersson J, Nordström EA, Blomqvist L, Sjögren A, Forsgren M, Attersand A, Arner P. A unique role of monocyte chemoattractant protein 1 among chemokines in adipose tissue of obese subjects. *J Clin Endocrinol Metab.* 2005 Oct;90(10):5834-40. Epub 2005 Aug 9.

(28) Delaigle AM, Jonas JC, Bauche IB, Cornu O, Brichard SM. Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. *Endocrinology.* 2004 Dec;145(12):5589-5597.

- (29) Dumond H. Evidence for a key role of leptin in osteoarthritis. *Arthritis Rheum.* 2003 Nov;48(11):3118-29
- (30) Duri ZA, Aichroth PM, Dowd G. The fat pad. Clinical observations. *Am J Knee Surg.* 1996 Spring;9(2):55-66.
- (31) Eger W, Schumacher BL, Mollenhauer J, Kuettner KE, Cole AA. Human knee and ankle cartilage explants: catabolic differences. *J Orthop Res* 2002 May;20(3):526-34.
- (32) Ehling A, Schaeffler A, Herfarth H, Tarner IH, Anders S, Distler O, Paul G, Distler J, Gay S, Schölmerich J, Neumann E, Müller-Ladner U. The potential of adiponectin in driving arthritis. *J Immunol.* 2006 Apr 1;176(7):4468-78.
- (33) Engeli S, Feldpausch M, Gorzelniak K, Hartwig F, Heintze U, Janke J, Möhlig M, Pfeiffer AF, Luft FC, Sharma AM. Association between adiponectin and mediators of inflammation in obese women. *Diabetes.* 2003 Apr;52(4):942-7.
- (34) Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology.* 2004 May;145(5):2273-82. Epub 2004 Jan 15.
- (35) Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest.* 2002 Oct;110(8):1093-1103.
- (36) Felson DT. An update on the pathogenesis and epidemiology of osteoarthritis. *Radiol Clin North Am.* 2004 Jan;42(1):1-9
- (37) Felson DT, Zhang Y, Hannan MT, Naimark A, Weissman BN, Aliabadi P, Levy D. The incidence and natural history of knee osteoarthritis in the elderly: the Framingham osteoarthritis study. *Arthritis Rheum* 1995 Oct;38(10):1500-1505.
- (38) Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. *Biorheology.* 2002;39(1-2):237-46.
- (39) Festa A, D'Agostino R Jr, Williams K, Karter AJ, Mayer-Davis EJ, Tracy RP, Haffner SM. The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes Relat Metab Disord.* 2001 Oct;25(10):1407-15.
- (40) Figenschau Y, Knutsen G, Shahazeydi S, Johansen O, Sveinbjörnsson B. Human articular chondrocytes express functional leptin receptors. *Biochem Biophys Res Commun.* 2001 Sep 14;287(1):190-7.
- (41) Flier, JS. Obesity wars: molecular progress confronts an expanding epidemic. *Cell.* 2004 Jan 23;116(2):337-350.
- (42) Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther.* 2006;8 Suppl 2:S3. Epub 2006 Jul 28.
- (43) Gabay O, Hall DJ, Berenbaum F. Osteoarthritis and obesity: experimental models. *Joint Bone Spine.* 2008 Dec;75(6):675-9. Epub 2008 Nov 20.
- (44) Gallagher J, Tierney P, Murray P, O'Brien M. The infrapatellar fat pad: anatomy and clinical correlations. *Knee Surg Sports Traumatol Arthrosc.* 2005 May;13(4):268-72. Epub 2005 Jan 28.

- (45) Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA Jr, Luster AD, Luscinskas FW, Rosenzweig A. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature*. 1999 Apr 22;398(6729):718-723
- (46) Gordeladze JO, Reseland JE. A unified model for the action of leptin on bone turnover. *J Cell Biochem*. 2003 Mar 1;88(4):706-12.
- (47) Graves DT, Jiang Y. Chemokines, a family of chemotactic cytokines. *Crit Rev Oral Biol Med*. 1995;6(2):109-18.
- (48) Griffin TM, Guilak F. Why is obesity associated with osteoarthritis? Insights from mouse models of obesity. *Biorheology*. 2008;45(3-4):387-98.
- (49) Guerre-Millo M. Adipose tissue and adipokines: for better or worse. *Diabetes Metab*. 2004;30:13-19
- (50) Hauner H. The new concept of adipose tissue function. *Physiol Behav*. 2004 Dec 30;83(4):653-8.
- (51) Henrotin Y, Deby-Dupont G, Deby C, De Bruyn M, Lamy M, Franchimont P. Production of active oxygen species by isolated human chondrocytes. *Br J Rheumatol* 1993;32:562.
- (52) Hochberg MC, Lethbridge-cejku M, Scott WW Jr, Reichle R, Plato CC, Tobin JD. The association of body weight, body fatness and body fat distribution with osteoarthritis of the knee: data from the Baltimore Longitudinal Study of Aging. *J Rheumatol*. 1995 Mar;22(3):488-93.
- (53) Hoffa A. The influence of the adipose tissue with regard to the pathology of the knee joint. *JAMA* 1904;42:795-796
- (54) Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006 Dec 14;444(7121):860-7.
- (55) Hutton CW. Hypothesis. Generalised osteoarthritis: an evolutionary problem? *Lancet* 1987 Jun 27;1(8548):1463– 5.
- (56) Kamei N, Tobe K, Suzuki R, Ohsugi M, Watanabe T, Kubota N, Ohtsuka-Kowatari N, Kumagai K, Sakamoto K, Kobayashi M, Yamauchi T, Ueki K, Oishi Y, Nishimura S, Manabe I, Hashimoto H, Ohnishi Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Nagai R, Kadowaki T. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *J Biol Chem*. 2006 Sep 8;281(36):26602-14. Epub 2006 Jun 29.
- (57) Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest*. 2006 Jun;116(6):1494-505. Epub 2006 May 11.
- (58) Kaneko S, Satoh T, Chiba J, Ju C, Inoue K, Kagawa J. Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. *Cytokines Cell Mol Ther*. 2000 Jun;6(2):71-9.
- (59) Kaneyama K, Segami N, Nishimura M, Suzuki T, Sato J. Importance of proinflammatory cytokines in synovial fluid from 121 joints with temporomandibular disorders. *Br J Oral Maxillofac Surg*. 2002 Oct;40(5):418-23.

- (60) Kavanaugh A, Anakinra. Interleukin-1 receptor antagonist has positive effects on function and quality of life in patients with rheumatoid arthritis. *Adv Ther.* 2006 Mar-Apr;23(2):208-17.
- (61) Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: Relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. *Diabetes* 2003;52:1779-1785
- (62) Kim CS, Park HS, Kawada T, Kim JH, Lim D, Hubbard NE, Kwon BS, Erickson KL, Yu R. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *Int J Obes (Lond).* 2006 Sep;30(9):1347-55. Epub 2006 Mar 14.
- (63) Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, Heyse SP, Hirsch R, Hochberg MC, Hunder GG, Liang MH, Pillemer SR, Steen VD, Wolfe F. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* 1998 May, 41(5):778-799.
- (64) Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet.* 2002 Jul 6;360(9326), 57-8.
- (65) Loeser RF. Systemic and local regulation of articular cartilage metabolism: where does leptin fit in the puzzle? *Arthritis Rheum.* 2003 Nov;48(11):3009-12
- (66) Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM. Leptin regulates proinflammatory responses. *FASEB J.* 1998 Jan;12(1):57-65.
- (67) MacConaill MA. The movements of bones and joints; the synovial fluid and its assistants. *J Bone Joint Surg Br.* 1950 May ;32B(2):244-252
- (68) Maccoux LJ, Salway F, Day PJ, Clements DN. Expression profiling of select cytokines in canine osteoarthritis tissues. *Vet Immunol Immunopathol.* 2007 Jul 15;118(1-2):59-67. Epub 2007 Apr 19.
- (69) Magi M, Branca C, Langerame V. Hoffa disease. *Ital J Orthop Traumatol.* 1991 Jun;17(2):211-6.
- (70) Malik KF; Young WS 3rd. Localization of binding sites in the central nervous system for leptin (OB protein) in normal, obese (ob/ob), and diabetic (db/db) C57BL/6J mice. *Endocrinology.* 1996 Apr;137(4):1497-1500
- (71) Manek NJ, Hart D, Spector TD, MacGregor AJ. The association of body mass index and osteoarthritis of the knee joint. An examination of genetic and environmental influences. *Arthritis Rheum.* 2003 Apr;48(4):1024-9.
- (72) Marks R. Obesity profiles with knee osteoarthritis: correlation with pain, disability, disease progression. *Obesity (Silver Spring).* 2007 Jul;15(7):1867-74.
- (73) Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol.* 2002 Aug;147(2):173-80.
- (74) McAlindon TE, Felson DT, Zhang Y, Hannan MT, Aliabadi P, Weissman B, Rush D, Wilson PW, Jacques P. Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. *Ann*

Intern Med. 1996 Sep 1;125(5):353-9.

(75) McAlindon TE, Wilson WF, Aliabadi P, Weissman B, Felson DT. Level of physical activity and the risk of radiographic and symptomatic knee osteoarthritis in the elderly: the Framingham study. *Am J Med* 1999 Feb;106(2):151-7.

(76) Miner JL. The adipocyte as an endocrine cell. *J Anim Sci*. 2004 Mar;82(3):935-41

(77) Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppel SW. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab*. 1997 Dec;82(12):4196-200.

(78) Moos V, Sieper J, Herzog V, Müller BM. Regulation of expression of cytokines and growth factors in osteoarthritic cartilage explants. *Clin Rheumatol*. 2001;20(5):353-8

(79) Neumeier M, Weigert J, Schäffler A, Wehrwein G, Müller-Ladner U, Schölmerich J, Wrede C, Buechler C. Different effects of adiponectin isoforms in human monocytic cells. *J Leukoc Biol*. 2006 Apr;79(4):803-8. Epub 2006 Jan 24.

(80) Oliveria SA, Felson DT, Cirillo PA, Reed JI, Walker AM. Body weight, body mass index, and incident symptomatic osteoarthritis of the hand, hip and knee. *Epidemiology*. 1999 Mar;10(2):161-6.

(81) Oliveria SA, Felson DT, Klein RA, Reed JI, Walker AM. Estrogen replacement therapy and the development of osteoarthritis. *Epidemiology* 1996;7:415-419.

(82) Otero M, Lago R, Lago F, Reino JJ, Gualillo O. Signalling pathway involved in nitric oxide synthase type II activation in chondrocytes: synergistic effect of leptin with interleukin-1. *Arthritis Res Ther* 2005;7(3):R581-R591. Epub 2005 Mar 4.

(83) Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*. 1999 Dec 21-28; 100(25):2473-6.

(84) Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. *J Biol Chem*. 2003 Mar 14; 278(11):9073-85.

(85) Pelletier JP, Martel-Pelletier J. Role of synovial inflammation, cytokines and IGF-1 in the physiopathology of osteoarthritis. *Rev Rhum Ed Fr*. 1994 Nov 15;61(9 Pt 2):103S-108S.

(86) Pottie P, Presle N, Terlain B, Netter P, Mainard D, Berenbaum F. Obesity and osteoarthritis: more complex than predicted! *Ann Rheum Dis*. 2006 Nov;65(11):1403-5.

(87) Presle N, Pottie P, Dumond H, Guillaume C, Lapique F, Pallu S, Mainard D, Netter P, Terlain B. Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production. *Osteoarthritis Cartilage*. 2006 Jul;14(7):690-5. Epub 2006 Mar 9.

(88) Rajala MW, Scherer PE. Minireview: The adipocyte-at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology*. 2003 Sep;144(9):3765-73.

(89) Ronti T, Lupattelli G, Mandarino E. The endocrine function of adipose tissue: an update. *Clin Endocrinol (Oxf)*. 2006 Apr;64(4):355-65

(90) Samantha A, Jones A, Regan M. Is osteoarthritis in women affected by hormonal change or smoking? *Br J Rheumatol* 1993;32:366-370.

- (91) Schaeffler A, Ehling A, Neumann E, Herfarth H, Tarner I, Schölmerich J, Müller-Ladner U, Gay S. Adipocytokines in synovial fluid. *JAMA*. 2003 Oct 1;290(13):1709-10.
- (92) Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D Jr. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Med*. 1996 May;2(5):589-93.
- (93) Sharma L. Local factors in osteoarthritis. *Curr Opin Rheumatol*. 2001 Sep;13(5):441-6
- (94) Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab*. 2007 Mar;92(3):1023-33. Epub 2006 Dec 12
- (95) Smith MD, Triantafyllou S, Parker A, Youssef PP, Coleman M. Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. *J Rheumatol*. 1997 Feb;24(2):365-71.
- (96) Sowers M. Epidemiology of risk factors for osteoarthritis: systemic factors. *Curr Opin Rheumatol*. 2001 Sep;13(5):447-51
- (97) Sowers MF, Lachance L. Vitamins and arthritis: the roles of Vitamins A,C,D and E. *Rheum. Dis Clin North Am*. 1999;25:315-332.
- (98) Spector TD, Dacre JE, Harris RA, Huskisson EC. Radiological progression of osteoarthritis: an 11 year follow up study of the knee. *Ann Rheum Dis* 1992 Oct; 51(10):1107–1110.
- (99) Spector TD, Hart DJ, Doyle DV. Incidence and progression of osteoarthritis in women with unilateral knee disease in the general population: the effect of obesity. *Ann Rheum Dis*. 1994 Sep; 53(9):565–568.
- (100) Spranger J, Verma S, Gohring I, Bobbert T, Seifert J, Sindler AL, Pfeiffer A, Hileman SM, Tschöp M, Banks WA. Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. *Diabetes*. 2006 Jan; 55(1):141-147.
- (101) Stefan N, Haering HU, Stumvoll M. Regulation of synovial adipocytokines. *JAMA*. 2004 Feb 11;291(6):694-5; author reply 695.
- (102) Stürmer T, Guenther KP, Brenner H. Obesity, overweight and patterns of osteoarthritis: the Ulm Osteoarthritis Study. *J Clin Epidemiol*. 2000 Mar 1;53(3):307-13.
- (103) Teichtahl AJ, Wluka AE, Proietto J, Cicuttini FM. Obesity and the female sex, risk factors for knee osteoarthritis that may be attributable to systemic or local leptin biosynthesis and its cellular effects. *Med Hypotheses*. 2005;65(2):312-5.
- (104) Terlain B. Leptin: a link between obesity and osteoarthritis? *Bull Acad Natl Med*. 2006 Oct;190(7):1421-35.
- (105) Thumb N. Function of cytokines in cartilage degradation. *Wien Med Wochenschr*. 1995;145(5):99-100.
- (106) Toda Y, Toda T, Takemura S, Wada T, Morimoto T, Ogawa R. Change in body fat, but not body weight or metabolic correlates of obesity, is related to symptomatic relief of obese patients with knee osteoarthritis after a weight control program. *J Rheumatol*. 1998 Nov;25(11):2181-6
- (107) Trayhurn P. Endocrine and signalling role of adipose tissue: new perspectives on fat. *Acta Physiol Scand*. 2005 Aug;184(4):285-93

- (108) Trayhurn P, Bing C, Wood IS. Adipose tissue and adipokines—energy regulation from the human perspective. *J Nutr.* 2006 Jul;136(7 Suppl):1935S-1939S
- (109) Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue, *Br J Nutr.* 2004 Sep;92(3):347-55
- (110) Ushiyama T, Chano T, Inoue K, Matsusue Y. Cytokine production in the infrapatellar fat pad: another source of cytokines in knee synovial fluids. *Ann Rheum Dis.* 2003 Feb;62(2):108-12.
- (111) van Beuningen HM, Glansbeek HL, van der Kraan PM, van den Berg WB. Osteoarthritis-like changes in the murine knee joint resulting from intra-articular transforming growth factor-beta injections. *Osteoarthritis Cartilage.* 2000 Jan;8(1):25-33.
- (112) van Harmelen, Skurk T, Röhrig K, Lee YM, Halbleib M, Aprath-Husmann I, Hauner H. Effect of BMI and age on adipose tissue cellularity and differentiation capacity in women. *Int J Obes Relat Metab Disord.* 2003 Aug;27(8):889-95
- (113) Vuolteenaho K, Koskinen A, Kukkonen M, Nieminen R, Päivärinta U, Moilanen T, Moilanen E. Leptin enhances synthesis of proinflammatory mediators in human osteoarthritic cartilage--mediator role of NO in leptin-induced PGE2, IL-6, and IL-8 production. *Mediators Inflamm.* 2009;2009:345838. Epub 2009 Aug 13
- (114) Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest.* 2003 Dec;112(12):1796-808.
- (115) Westacott CI, Sharif M. Cytokines in osteoarthritis: mediators or markers of joint destruction? *Semin Arthritis Rheum.* 1996 Feb;25(4):254-72.
- (116) Wisse BE. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. *J Am Soc Nephrol.* 2004 Nov;15(11):2792-800
- (117) Yoda-Murakami M, Taniguchi M, Takahashi K, Kawamata S, Saito K, Choi-Miura NH, Tomita M. Change in expression of GBP28/adiponectin in carbon tetrachloride-administrated mouse liver. *Biochem. Biophys. Res. Commun.* 2001 Jul 13;285(2): 372–377.
- (118) Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood.* 2000 Sep 1;96(5):1723-32.
- (119) Yudkin JS. Adipose tissue, insulin action and vascular disease: inflammatory signals. *Int J Obesity Rel Meatab Diord.* 2003 Dec; 27 Suppl. 3 S:25-S28.
- (120) Yudkin JS, Eringa E, Stehouwer CD. 'Vasocrine' signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. *Lancet.* 2005 May 21-27;365(9473): 1817–1820.
- (121) Zozulinska D, Majchrzak A, Sobieska M, Wiktorowicz K, Wierusz-Wysocka B. Serum interleukin-8 level is increased in diabetic patients. *Diabetologia.* 1999 Jan;42(1):117-118.
- (122) www.copewithcytokines.de

8. List of Tables

Image 1: Sagittal demonstration of the knee joint showing the location of the IFP. Elsevier 2006. F.H. Netter: Atlas of Human Anatomy.....	7
Image 2: Axial demonstration of the knee joint showing the location of the IFP. Elsevier 2006. F.H. Netter: Atlas of Human Anatomy.....	7
Image 3: Trayhurn, Bing C, Wood IS. Adipose tissue and adipokines—energy regulation from the human perspective. J Nutr. 2006 Jul;136(7 Suppl):1935S-1939S.....	16
Figure 1: Mean volumes of IFP (H) and subcutaneous control adipocytes (C).....	33
Figure 2: IL-6-release from 1 mio. adipocytes of subcutaneous adipose tissue of the thigh (C) and 1 mio. cells of the IFP (H).....	35
Figure 3: IL-6-release from 100mg tissue of subcutaneous fat (C), the IFP (H) and synovial membrane (S).....	35
Figure 4: Correlation between IL-6-secretion of 1 mio. cells of the IFP (H) and age of patients.....	36
Figure 5: IL-8-release from 1 mio. adipocytes from subcutaneous adipose tissue (C) and 1 mio. cells from the IFP (H).....	37
Figure 6: IL-8-release of 100mg tissue from subcutaneous fat (C), the IFP (H) and synovial membrane (S).	37
Figure 7: Correlation between the IL-8-release of 1 mio. cells of the IFP (H) and age of patients.....	38
Figure 8: TNF- α -release from 1 mio. adipocytes of subcutaneous fat (C) and from 1 mio. cells of the IFP (H).	39
Figure 9: TNF- α -release from 100mg tissue of subcutaneous fat (C), the IFP (H) and synovial membrane (S).....	39
Figure 10 : TNF-alpha-secretion of 100mg tissue of the IFP (H) in Women (W) and in Men (M).....	40
Figure 11: Correlation between TNF- α -production in 100mg tissue of synovial membrane (S) and 1 mio. cells of the IFP (H).....	40
Figure 12: Correlation between TNF- α -production of 100mg tissue of synovial membrane (S) and 100mg tissue of the IFP (H).....	41
Figure 13: Correlation between TNF- α -production of 100mg tissue of synovial membrane (S) and from 1 mio. cells of subcutaneous fat (C).	41

Figure 14: Correlation between TNF- α -production of 100mg tissue of synovial membrane (S) and 100mg tissue of subcutaneous fat (C)	42
Figure 15: MCP-1-release from 1 mio. adipocytes of subcutaneous fat (C) and 1 mio. cells of the IFP (H).....	43
Figure 16: MCP-1-release from 100mg tissue subcutaneous fat (C), the IFP (H) and synovial membrane (S).....	43
Figure 17: Adiponectin-release from 1 mio. adipocytes of subcutaneous fat (C) and of 1 mio. cells of the IFP (H).....	44
Figure 18: Adiponectin-release from 100mg tissue of the IFP (H), subcutaneous fat (C) and synovial membrane (S)	45
Figure 19: Correlation between adiponectin-production in 1 mio. cells of the IFP (H) and the Volume of the IFP (H).....	45
Figure 20: Correlation between adiponectin-production in 1 mio. adipocytes of subcutaneous fat (C) and Weight of the IFP (H).	46
Figure 21: Leptin-release from 1 mio. adipocytes of subcutaneous fat (C) and from 1 mio. cells of the IFP (H).....	47
Figure 22: Leptin-release from 100mg tissue of subcutaneous fat (C), the IFP (H) and synovial membrane (S).	48
Figure 23: Correlation between leptin-production of 1 mio. adipocytes of the IFP (H) and adipocyte volume of the IFP (H).....	48
Figure 24: Correlation between leptin-production in 100mg tissue of the IFP (H) and the body mass index (BMI).....	49
Figure 25: Correlation between leptin-production in 1 mio. cells of subcutaneous fat (C) and the adipocyte volume of subcutaneous fat (C)	49
Figure 26: Correlation between leptin-release of synovial membrane (S) and the adipocyte volume of subcutaneous fat (C).....	50
Figure 27: Correlation between leptin-production of the synovial membrane (S) and leptin-production in 1 mio. cells of subcutaneous fat (C).....	50
Figure 28: Correlation between leptin-production in synovial membrane (S) and leptin-production in 100 mg tissue of subcutaneous fat (C)	51
Figure 29: Amount of IL-6, IL-8, TNF- α , MCP-1, adiponectin and leptin found in Synovial Fluid (SYN).....	52
Figure 30: Correlation between amount of leptin measured in synovial fluid (SYN) and adipocyte volume of subcutaneous fat (C)	52

9. List of Abbreviations

ACL	Anterior cruciate ligament
BMI	Body mass index
bFGF	Basic fibroblast growth factor
C	Control fat from the thigh
CRP	C-reactive protein
H	Hoffa fat pad
HIF-1	Hypoxia-inducible factor 1
IL-1	Interleukin 1
IL-4	Interleukin 4
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-1RA	Interleukin 1 receptor antagonist
MCP-1	Monocyte chemoattractant protein 1
MetS	Metabolic syndrome
NPY	Neuropeptide Y
OA	Osteoarthritis
PG	Proteoglycan
S	Synovial membrane
IFP	Infrapatellar fat pad
RA	Rheumatoid arthritis
SCF	Subcutaneous fat from the thigh
SYN	Synovial fluid
TNF- α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor
WAT	White adipose tissue

10. Acknowledgements

I would like to thank Prof. Dr. med. R. Gradinger and Prof. Dr. med. H. Hauner for giving me the opportunity to work on this very interesting project.

Many thanks as well to PD Dr. med. R. Burgkart, Dr. med. T. Skurk and Dr. med. H. Pilge for their supervision and their advice, which I am very grateful for.

Moreover, I would like to thank all of my co-workers at the Department of Orthopaedic Surgery and at the Department for Nutritional Medicine in Weihenstephan for their patient guidance.

And in particular, I thank my parents, my sister and all of my friends for their outstanding support.