SYNOVIAL METABOLISM AFTER DIFFERENT DEGREES OF TRAUMA. EFFECTS OF PHARMACOLOGICAL AND PHYSIOLOGICAL INTERVENTION

A MICRODIALYSIS STUDY OF THE KNEE JOINT SYNOVITIS

Anders Stålman
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Anders Stålman

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Cover: Arthroscopically assisted insertion of the microdialysis catheter in the inner layer of the knee joint capsule; the synovial membrane

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Gårdsvägen 4, 169 70 Solna
To my family
Abstract

Background

Knee arthroscopy is one of the most common orthopedic procedures. Postoperative pain and knee swelling is often a problem. Optimal pain management in knee arthroscopic procedures is important to permit same-day discharge and to contribute to early mobilisation, reduced cost and increased patient satisfaction. The process of nociception, inflammation and postoperative pain perception is not fully understood. Pre-, intra- and postoperative treatment may be improved by increasing the knowledge regarding local metabolic and inflammatory response in the knee synovium.

Aim and Hypothesis

The aim of these studies were to monitor local changes in classic metabolic compounds and PGE_{2} in relation to the magnitude of surgical trauma and pain subjectively experienced by the patient and to analyze the local effects of intervention with commonly used intra-articular medication and postoperative cryotherapy. Our hypothesis was that the degree of surgical trauma and postoperative pain as experienced by the patient would be reflected by disturbances in synovial metabolism and inflammatory reactions. We further hypothesised that intra-articular administration of morphine, ketorolac or postoperative application of cryotherapy would be reflected by decreased disturbances in expression of classic metabolic compounds and inflammatory reactions.

Material and Methods

In total 130 patients completed the studies. Inclusion criteria were indication for arthroscopy due to signs of meniscus injury (Study 1-4) or a symptomatic ACL injury requiring reconstruction (Study 2). In study 1 patients were divided in two groups; NO need of rescue medication (n=8) and need for rescue medication (n=6). In study 2; 20 patients undergoing knee arthroscopy were compared to 10 patients undergoing ACL reconstruction. In study 3; 60 patients were randomised into three equal subgroups; intra-articular administration of morphine, ketorolac (and placebo). In study 4; 40 patients were randomised into two equal subgroups; application of cooling and compression and NO application of cooling and compression. Synovial metabolism and inflammatory reaction was monitored by microdialysis of glucose, lactate, glycerol, PGE_{2} (study 1-4) and glutamate (study 3-4). Local temperature; intra-articular, in the joint capsule and on skin were monitored in study 4.

Results

Study 1: Higher PGE_{2} levels that decreased over time and consumption of glucose were found in patients experiencing more postoperative pain. Study 2: In comparison with the knee arthroscopy group the ACL group showed lower levels of PGE_{2} and less lactate increase and glucose consumption. Study 3: There were no apparent effects of morphine but ketorolac significantly reduced PGE_{2} levels. Study 4: Less lactate increase was noted in the cooling and compression group. Expression of PGE_{2} was temperature sensitive.

Discussion

Pain after arthroscopy was reflected by increased glucose utilization and PGE_{2} production by the synovial membrane (study 1). This indicates that there is a state of hypermetabolism in patients experiencing pain and that PGE_{2} is a pain marker. ACL reconstruction did not influence the local synovial metabolism or induce an inflammatory response at the same degree as seen after knee arthroscopy (study 2). Intra-articular injection of morphine and/or the use of a combined cooling and compression device in the ACL group may have influenced the findings. Intra-articular injection of ketorolac significantly lowered PGE_{2} levels but no apparent effects for morphine were seen (study 3). This is in agreement with the known effects of prostaglandin synthesis inhibitors, NSAIDs, on prostaglandins and pain. Local cryotherapy after knee arthroscopy significantly lowered knee temperature postoperatively. A correlation with synovial PGE_{2}and temperature was found. Hypothermia blunted the previously shown lactate increase (study 4). This implicates a positive anti-inflammatory effect induced by postoperative local cooling and compression. Hypothermia is proposed to have a tissue protective effect during reperfusion by means of a decreased metabolic rate.
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1 LIST OF ABBREVIATIONS

ACL  Anterior cruciate ligament
AMPA  $\alpha$-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
ATP  Adenosine triphosphate
COX 1 and 2  Cyclooxygenase 1 and 2
CSF  Cerebrospinal fluid
ECF  Extracellular fluid
NADPH  Nicotinamide adenine dinucleotide phosphate
NMDA  N-methyl-D-aspartic acid
NRS  Numeric Rating Scale
NSAID  Non steroidal anti inflammatory drugs
OA  Osteoarthritis
PGE$_2$  Prostaglandin E$_2$
VAS  Visual Analogue Scale
2 LIST OF PUBLICATIONS

Högberg E, Stålman A, Wredmark T, Tsai JA, Arner P, Felländer-Tsai L.

**Opioid requirement after arthroscopy is associated with decreasing glucose levels and increasing PGE2 levels in the synovial membrane.**

Stålman A, Tsai JA, Wredmark T, Dungner E, Arner P, Felländer-Tsai L.

**Local inflammatory and metabolic response in the knee synovium after arthroscopy or arthroscopic anterior cruciate ligament reconstruction.**

Stålman A, Tsai JA, Segerdahl M, Dungner E, Arner P, Felländer-Tsai L.

**Ketorolac but not morphine exerts inflammatory and metabolic effects in synovial membrane after knee arthroscopy: a double-blind randomized prospective study using the microdialysis technique.**

Stålman A, Berglund L, Dungner E, Arner P, Felländer-Tsai L.

**Temperature sensitive release of PGE2 and diminished energy requirements in synovial tissue with postoperative cryotherapy: a prospective randomized study after knee arthroscopy.**
Submitted

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3 INTRODUCTION

3.1 BACKGROUND

Knee arthroscopy is a common method in diagnosing and treating intra-articular knee pathology. It is one of the most common orthopedic procedures. In Sweden approximately 40,000 arthroscopic procedures in the knee are performed every year. The procedure can be done safely as ambulatory surgery in general-, spinal- or local anesthesia (Tsai and Wredmark 1993; Jacobson, Forssblad et al. 2000). Postoperative pain and knee swelling is common and often requires consumption of analgesics (Rockborn, Hamberg et al. 2000; Jacobson, Assareh et al. 2006). Optimal pain management with knee arthroscopic procedures is important to enable same-day discharge and to contribute to early mobilization, reduced cost and increased patient satisfaction. The process of inflammation, nociception and postoperative pain perception is not fully understood. Pre-, per- and postoperative treatment may be improved by increasing the knowledge of the local metabolic and inflammatory response in the synovium.

3.2 SYNOVIAL JOINT

The joint cavity is enclosed by a two-layer articular capsule, or joint capsule. The external layer is a fibrous capsule, that is continuous with the periostea of the articulating bones (stratum fibrosum). The inner layer of the joint capsule is a secreting layer (stratum synoviale) described as the synovial membrane. The synovial membrane is a crucial metabolically active tissue producing the viscous, egg-white consistency fluid, synovial fluid (synovi = egg latin) containing lubricants and nutrients for the joint. The synovial membrane is richly vascularized and folded into the joint so that in any position the cartilage covering the bony surfaces is close enough to get nutrition directly from the synovium (figure 1).

Figure 1. Synovial joint
Trauma or pathologic conditions such as inflammatory disease or degenerative joint disease affects synovial metabolism and can be deleterious to joint cartilage and further impair joint function. Inflamed synovium produce excess synovial fluid which can provide a barrier to diffusion of nutrients to cartilage. The inflamed synovium also utilises nutrients leading to cartilage starvation (Edwards J CW. 1998). Joints with active arthritis are rich in inflammatory markers and excitatory amino acids (Lawand, McNearney et al. 2000; McNearney, Speegle et al. 2000; Averbeck, Rudolphi et al. 2004). The synovial fluid has less lubricating ability in joint arthritis or after joint trauma (Elsaid, Jay et al. 2005; Elsaid, Fleming et al. 2008) and knee joint arthroscopy imposes metabolic changes to the synovium (Fellander-Tsai, Hogberg et al. 2002).

3.3 ARTHROSCOPY

3.3.1 History

History of arthroscopy reaches back to 1879 when Nitze (1848-1706) constructed the first cystoscope and demonstrated that it was possible to perform surgery through this instrument. Since then several physicians have contributed to the development of the technology and surgical technique, among them the Swedish physician Jacobsen who developed the "laparo-thoracoscope" which was used for treatment of pleural adhesions caused by tuberculosis. The term "arthroscopy" was first seen in a manuscript presented in 1912 by the Danish surgeon Nordentoft and he was probably the first surgeon to apply endoscopic technique to the knee joint. Takagi (1888-1963) further developed the technique and also discussed distention of the knee with saline solution to increase the size of the joint cavity and enable better visualisation. Bircher (1882-1956) became the first to publish findings of meniscus pathology and posttraumatic arthritis when describing his findings in patients 1921. The method was met with skepticism and it wasn’t until 1962 when Takagi’s student Watanabe described the first successful arthroscopic partial meniscus resection. This was a milestone in orthopedic surgery and a significant advancement to open total meniscectomy commonly performed since the frequency of osteoarthritic radiographic changes after meniscectomy is related to the size of the meniscus removed (Andersson-Molina, Karlsson et al. 2002). Watanabe further developed instruments similar to the ones we use today, introduced operative techniques and published the Atlas of Arthroscopy (Jackson 2010).
3.3.2 *Surgery of the meniscus*

Pain in the knee and symptoms of swelling and locking due to meniscus injuries is probably the most common indication for knee arthroscopy. The meniscus is a fibrocartilaginous crescent-shaped disc which acts to disperse the weight of the body in the joint during movement. Menisectomy increases the risk of developing osteoarthritis of the knee (Roos, Lauren et al. 1998). The extent of resection relates to the degree of radiologic osteoarthritis (Englund and Lohmander 2004). This has increased the interest of meniscal repair. Several techniques of meniscal repair have evolved but no significant difference in clinical outcome between the different techniques has been shown. A number of factors affect healing after meniscal repair. Negative prognostic factors are older patients, large distance from the vascularized periphery, ligament instability and complex or degenerative tears (Starke, Kopf et al. 2009).

3.3.3 *Anterior cruciate ligament reconstruction (ACL)*

The ACL is composed of two distinct bundles – the anteromedial and the posterolateral, named according to where the bundles insert into the tibial plateau. The ACL is controlling anterior translation and medial rotation of the tibia in relation to the femur. The posterolateral bundle is tense in full extension and the anteromedial bundle has its maximal tension in 60 degrees of flexion (Petersen and Zantop 2007).

Rupture of the ACL is a common and serious knee injury. The incidence of this injury in Sweden is reported to be 71/100 000 and 32/100 000 undergo surgical reconstruction each year. Playing soccer is the most common cause of injury comprising 41 % of the ACL injuries and the risk is greater in female athletes. 35 % of the patients with an ACL injury also present meniscus injuries and 27 % present cartilage injuries (Granan, Forssblad et al. 2009).

The ACL injured patient often presents with acute hemarthrosis and pain. Non-operative management of ACL injuries is probably sufficient in the absence of subjective instability in activities of daily life and if a restricted activity level is tolerated (Muaidi, Nicholson et al. 2007; Neuman, Englund et al. 2008). If surgery is indicated an arthroscopic assisted reconstruction with either bone-patellar tendon-bone or hamstring tendon autograft is undertaken. The question whether one graft is superior to the other is unanswered but the patients seem to experience more anterior knee pain if patellar tendon graft is used (Kartus, Mavin et al. 2001; Herrington, Wrapson et al. 2005). Another option is allografts but it should be remembered that graft incorporation and ligamentization are probably slower for allografts compared with autografts. Allografts are more vulnerable to failure and require more careful rehabilitation (Kuhn and Ross 2007). We reserve allografts for revision surgery or multiligamentous injuries in patients over age 30 without high activity demands. The risk of developing osteoarthritis is high after an ACL injury regardless of reconstruction or not and the risk increases if the injury is associated with a meniscus tear (Louboutin, Debarge et al. 2009).
3.3.4 Osteoarthritis (OA)

Osteoarthritis (OA) of the knee is a common disorder that increases with age. Symptomatic knee joint arthritis affect approximately 12% of the population >60 years (Lawrence, Felson et al. 2008). OA gives rise to considerable morbidity for the individual with decreased quality of life and also substantial socioeconomic consequences with loss of productivity and high costs of healthcare (Kramer, Yelin et al. 1983; Rabenda, Manette et al. 2006). The etiology of OA is multifactorial and the mechanisms are poorly understood. OA can be idiopathic or secondary to trauma as in ACL or meniscus injury (Oiestad, Engebretsen et al. 2009). There is probably also a genetic susceptibility to OA (Valdes and Spector 2010). It is known that knee trauma and an ACL injury give rise to metabolic changes in the knee joint and changes in the composition of the synovial fluid (Jean, Wen et al. 2005; Elsaid and Chichester 2006; Elsaid, Fleming et al. 2008; Larsson, Lohmander et al. 2009). These changes in cartilage and synovial fluid composition may further impair joint function and contribute to disease progression. Knee surgery, such as knee arthroscopy also causes trauma to the knee synovium (Fellander-Tsai, Hogberg et al. 2002). It is not known whether the trauma caused by knee arthroscopy has an additative effect on the risk factors for degenerative knee disease.

The pathological process of OA probably starts many years before we see radiographic signs of osteoarthritis and before the patient experiences symptoms of pain. It is a challenge to find a genetic- or a bio-marker for early osteoarthritis to introduce preventive treatment or better disease management strategies. It is not known what part the different involved tissues: cartilage, bone or synovium play in the pathogenesis of OA. Several degradable products from cartilage, bone and synovium have been studied in search of a bio-marker of OA in hope to find disease modifying treatments. However, so far current medical treatment is limited to symptom relief (Hunter, Li et al. 2007; Dam, Loog et al. 2009).

Pain is the most important symptom for the individual with OA. But symptomatic pain is only weakly related to radiological or arthroscopic findings of joint destruction (Bedson and Croft 2008; Marticke, Hosselbarth et al. 2010). Knowledge of the pathogenesis of joint pain is important. Cartilage is aneural but periosteum, joint capsule and synovium is richly innervated and can be the source of nociceptive stimuli.

Peripheral and central pain sensitisation, in which the neurotransmitter glutamate plays a crucial role, is also a feature of OA (Schaible, Ebersberger et al. 2002). Treatment is first non-pharmacological with e.g exercise in adjusted activities or pharmacological (Creamer 2000). Today, the final step is knee replacement surgery.
3.4 PRE-, INTRA- AND POSTOPERATIVE MANAGEMENT

3.4.1 Bloodless field – Ischemia and reperfusion injury

Knee arthroscopy, particularly arthroscopically assisted ACL reconstruction, is commonly performed in a bloodless field to reduce intra-articular bleeding and improve vision. Using bloodless field in arthroscopically assisted knee surgery is probably a safe procedure even though risk factors such as nerve injuries, increased risk of deep venous thrombosis, knee pain and stiffness has been attributed to its use (Smith and Hing 2009).

Knee arthroscopy is performed under distention of the joint with irrigation fluid that could cause temporary ischemia by itself (Fellander-Tsai, Hogberg et al. 2002).

The ischemia/reperfusion injury process that leads to cell death and dysfunction is multifactorial and complicated. The first component is the lack of oxygen and conversion of the cellular metabolism to anaerobic pathways, ischemia. Hypoxia and energy depletion lead to malfunction of the cell membrane transport systems. The oxidative phosphorylation in the mitochondria is impaired. The anaerobic glycolytic pathway will remain the only available energy source. The cellular levels of adenosine triphosphate (ATP) will decrease. Active extrusion of sodium ions by the Na/K-ATP pump will be inhibited and sodium concentration in the cell increases, causing cell swelling. Intracellular calcium concentration is 1000 to 10000 times lower than that of extracellular fluid due to the action of ATP dependent calcium sequestering systems. Lack of ATP leads to increased intracellular calcium levels and calcium induces activation of phospholipases and proteases degrading phospholipids, which are required for the integrity of the cell membrane (Ar’Rajab, Dawidson et al. 1996). Phospholipases and proteases trigger processes which lead to formation and release of proinflammatory agents such as platelet activation factor, leukotrien, thromboxan and prostaglandin species. Neutrophil adhesion, emigration and degranulation of proteolytic enzymes is promoted. Prolonged ischemia will result in cell disintegration and ultimately cell death (Kerrigan and Stotland 1993).

The reestablishment of normal vascular supply can further damage the tissue, the reperfusion paradox. This is due to the formation of toxic oxygen free radicals. Ischemia induce breakdown of adenine nucleotides to hypoxanthine and conversion of xanthine dehydrogenase to oxygen free radical producing xanthine oxidase. Polymorphonuclear leukocytes accumulating in posts ischemic tissues contain membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. NADPH oxidase oxidizes cytoplasmic NADPH contributing to formation of oxygen free radicals (Korthuis and Granger 1993). Oxygen free radicals are reactive chemicals hazardous to cellular structures (Ar’Rajab, Dawidson et al. 1996).
3.4.2 Intra-articular administration of pharmacological substances

3.4.2.1 Opioids

Opioid receptors are found in peripheral nerve endings (Stein, Hassan et al. 1993). Intra-articular administration of opioids is used in knee arthroscopy to decrease postoperative pain and dysfunction but contradictory results on the effects have been shown (Gupta, Bodin et al. 2001; Rosseland 2005). The different results can have many reasons. A dose dependency, higher opioid dose giving better pain relief, has been suggested (Likar, Kapral et al. 1999). It has been proposed that administration of analgesic agents preoperatively, i.e. pre-emptive analgesia, could better prevent pain sensitisation and improve postoperative pain control (Denti, Randelli et al. 1997; Ong, Lirk et al. 2005). Opioid receptor up-regulation occurs in inflamed tissue and inflammation may be a prerequisite for peripheral opioid analgesia (Zollner, Shaqura et al. 2003).

Opioid-containing immune cells extravasate using adhesion molecules and chemokines to accumulate in inflamed tissues. Endogenous opioid peptides are secreted and produce analgesia by inhibiting the excitability of sensory nerves and the release of excitatory neuropeptides without central adverse effects such as depressing breathing and affecting consciousness (Machelska 2007). The major source of endogenous opioids (beta-endorphin, enkephalin, endomorphin and dynorphin) are leukocytes. The predominant opioid peptide involved in immune-cell mediated antinociception is thought to be beta-endorphin. The importance of endogenous opioids in peripheral analgesia is proven by the fact that impaired immune cell function as in immunosuppressive disease may increase inflammatory pain (Hermanussen, Do et al. 2004). Evidence for the expression of opioid receptors on immune cells suggest that endogenous and exogenous opioids can modulate immune cell function (Kapitzke, Vetter et al. 2005). Exogenous opioids may apart from their central adverse effects possibly incur negative effects on immune cell function adding further complexity (Risdahl, Khanna et al. 1998). Endogenous opioids on the other hand not only control pain but also stimulate immune function (Menzebach, Hirsch et al. 2003). This complex balance in the immune system is of great interest and a better understanding of the endogenous pathways involved in peripheral analgesia may enable us to target these pathways for antinociceptive approaches and possibly avoid systemic side effects of opioid treatment.

3.4.2.2 Non-Steroidal Anti-Inflammatory Drugs (NSAID)

Ketorolac, a non-steroidal anti-inflammatory drug (NSAID) seems to produce analgesia when administered intra-articularly either alone or in combination with local anesthetics or opioids (Gupta, Axelsson et al. 1999; Calmet, Esteve et al. 2004; Ng, Nordstrom et al. 2006). Prostaglandin E2 (PGE2) is involved in the process of peripheral and central pain sensitization (Vasquez, Bar et al. 2001; Schäible,
Ebersberger et al. 2002). High levels of PGE2 have been found in synovial fluid in inflammatory joint
disease and the concentration of PGE2 seems to correlate to the amount of pain (Trumble, Billinghurst et
al. 2004). The mechanism of action of NSAIDs is in part by inhibiting prostaglandin synthesis (Zhang,
Shaffer et al. 1997; Creamer 2000).

Adverse effects for NSAIDs on joint tissue have been proposed (Irwin, Cheung et al. 1998). Therapeutic
concentrations of non-selective NSAIDs seem to cause proliferation suppression and cell death of
chondrocytes but selective COX-2 NSAIDs show less deleterious effects on chondrocytic proliferation and
death (Chang, Wu et al. 2006). An intra-articular injection of the selective cyclooxygenase 2 inhibitor
parecoxib has even been shown to attenuate osteoarthritis progression and to reduce levels of glutamate
in synovial fluid (Jean, Wen et al. 2007). It is not known whether these findings have clinical significance
in man.

3.4.2.3 Local anesthetics

Despite widespread use of local anesthetics in knee arthroscopy there is little consensus on it regarding
optimal doses and in which surgical procedures local anesthetics give clinical relevant pain relief
(Moiniche, Mikkelsen et al. 1999). There is evidence for a postoperative moderate and short-lasting effect
of intra-articular local anesthetics (Chirwa, MacLeod et al. 1989). The effect is dose dependent and
possibly better if administered preoperatively (Gyrn, Olsen et al. 1992; Hube, Troger et al. 2009).

Intra-articular injection of 20-30 ml 0,25 - 0,75 % bupivacain resulted in atoxic serum levels and the peak
serum levels could be reduced by adding epinephrine and injection after tourniquet inflation (Wasudev,

There are several reports on the chondrotoxic effects of local anesthetics. Chondrocyte viability decreases
in a dose-depandent matter. There is probably a difference between different anesthetic agents with more
deleterious effects on cell viability for lidocaine and bupivacain and perhaps more favorable results for
ropivacain in vitro (Piper and Kim 2008; Grishko, Xu et al. 2010). A single intra-articular injection of 0,5 %
bupivacain showed subtle and subclinical toxic effects on cartilage in vivo (Chu, Coyle et al. 2010).
Continuous intra-articular bupivacain infusion in a rabbit model showed profound chondrotoxic effects,
but possibly with a reparative response (Gomoll, Kang et al. 2006; Gomoll, Yanke et al. 2009). Numerous
studies and case reports have shown glenohumeral joint chondrolysis in man after continuous bupivacain
infusion (Rapley, Beavis et al. 2009).

The toxicity of a single dose of local anesthetics may be of little clinical significance but it is a challenge to
find better alternatives of intra-articular analgesia. Perhaps subcutaneous portal infiltration with local
anesthetics is sufficient in knee arthroscopy of short duration performed in general anesthesia
(Townshend, Emmerson et al. 2009).
3.4.3 Application of a combined cooling and compression device

Cryotherapy is a common treatment paradigm for analgesia and enhanced recovery after arthroscopic surgery (Raynor, Pietrobon et al. 2005). It is an effective modality for decreasing intra-articular temperature after knee arthroscopy (Martin, Spindler et al. 2001). Cryotherapy treatment is often combined with compression and the effects of each modality is uncertain (Schroder and Passler 1994; Dervin, Taylor et al. 1998).

Hypothermia is known to retard cellular metabolism and thus serves to increase tissue tolerance to ischemia (Francel, Vander Kolk et al. 1992; Mowlavi, Neumeister et al. 2003).

Hypothermia also seems to have an anti-inflammatory action since there is a temperature sensitive action in the release of prostaglandins and inflammatory chemokines (Wang, Xuan et al. 1993; Diestel, Roessler et al. 2008). Hypothermia reduces the action of the enzymes phospholipase C and A2 crucial for prostaglandin biosynthesis (Majumdar, Gowda et al. 1995).

The tissue protective effects of mild to moderate (32-35 degrees C) hypothermia has been studied clinically in post-ischemic cardiac- and neurological injuries with promising results (Polderman 2009). Mild hypothermia may decrease the harmful effects of reperfusion in ischemic tissue (Kanemoto, Matsubara et al. 2009).
3.5 MICRODIALYSIS

Tissue microdialysis makes it possible to continuously monitor the concentration of molecules in the interstitial fluid (Ungerstedt 1991). It is an in vivo sampling technique used for study of local tissue-specific events. It can be used safely in humans with low grade of invasiveness and allows sampling over prolonged periods of time. Microdialysis is a useful tool in investigations of human metabolism in, e.g, monitoring tissue metabolism pre- and postsurgery in vascular and plastic surgery, in critical care medicine after multitrauma, sepsis or brain trauma or in studies of drug distribution, metabolism and pharmacodynamics (Rooyackers, Thorell et al. 2004; Stolle, Arpi et al. 2004; Setala, Papp et al. 2006; Wei, Xu et al. 2009; Birke-Sorensen and Andersen 2010).

3.5.1 History

The first study with a microdialysis technique was published 1966 when Bito and colleagues implanted "dialysis sacs" with semi-permeable membranes into the cortex and subcutaneously in the neck of dogs. Ten weeks later the contents of the sacs, representing the composition of the extracellular fluid (ECF), were analyzed for amino acids and ions and compared to the concentrations in cerebrospinal fluid (CSF) and plasma. They found a concentration gradient in the following order; plasma>ECF>CSF concluding that the brain ECF represents a third compartment and supporting the idea of a carrier-mediated transport system for amino acids. Their use of "dialysis sacs" allowed only one sample per animal and could not detect changes over time (Bito, Davson et al. 1966).

Delgado et al developed a cannula with a small semi-permeable membrane bag on its tip a 'dialytrode'. This made it possible to continuously sample endogenous compounds (Delgado, DeFeudis et al. 1972). They were able to continuously collect amino acids, metabolites and glycoproteins with intracerebral perfusion in awake rhesus monkeys. They demonstrated that perfusion of glutamate through the amygdala induced typical glutamate after-discharges and concluded that the system provides a new diagnostic and therapeutic procedure in man, to obtain neurochemical information from, and to deliver drugs to specific structures of the brain.

Ungerstedt and Pycock further developed the technique in their studies of dopamine neurotransmission with the use of a hollow fiber dialysis probe that eventually developed into the needle probe similar to the one we use today (Ungerstedt and Pycock 1974). Since then almost 13000 papers (pubmed.org) are published describing the use of microdialysis in pharmacology, physiology, clinical monitoring and other related biomedical fields. These studies refer basically to the same principle, probe design and experimental set-up as originally described by Ungerstedt and Pycock (1974).
3.5.2 Principles

The technique involves the implantation of a small probe, a catheter with a tubular semi-permeable dialysis membrane on its tip, into a specific region of a tissue or a fluid-filled space. Substances in the interstitial space diffuse across the semipermeable membrane to a physiologically compatible perfusion fluid (per fusate), which is pumped through the microdialysis catheter. The microdialysis catheter then mimics a capillary vessel (figure 2).

Figure 2. The microdialysis catheter mimics a capillary vessel (CMA Microdialysis).

The degree of equilibration between the interstitial space and the perfusion fluid (recovery) is mainly dependent on the perfusion flow, the length of the dialysis membrane and size of the pores in the semi permeable membrane.

In addition to perfusate flow rate and probe geometry, extraction fraction is influenced by a number of solute and tissue related factors. Among these factors are the physico-chemical properties of the solute of interest, its diffusion coefficient in the tissue and the processes for elimination from the tissue, including active transport mechanisms (Chaurasia, Muller et al. 2007).

Most often microdialysis is performed with an incomplete recovery representing relative changes of an unknown fraction of the interstitial concentration. If quantitative information on ECF is desired
calibration methods can be used. Absolute recovery can be acquired by using the low-flow-rate method or calibrated by the variation of concentrations (point of no-net-flux technique) and by varying flow rate, the zero-flow method (Kehr 1993).

Using the low-flow-rate method the perfusion flow rate for allowing near 100% recovery is 0,16 µl/min for glucose, 0,33 µl/min for lactate and 0,66 µl/min for glycerol with a 30 mm long membrane allowing passing of 20 kD molecules (CMA 60, CMA Microdialysis, Solna, Sweden) (Rosdahl, Hamrin et al. 1998). Low perfusion flow results in loss of perfusion fluid. High concentrations of macromolecules in the extracellular tissue tend to dilute the compartment and perfusate will be lost. Almost 50% of the perfusion fluid is lost in adipose tissue and in muscle near 10% at 0,16 µl/min (Rosdahl, Hamrin et al. 1998). To make samplings at low perfusion flows possible, without a substantial loss of fluid to the tissue, a colloid needs to be included in the perfusion fluid. It may be expected that the addition of a colloid to the perfusion fluid would significantly decrease metabolite concentrations in the microdialysis samples by increasing the effective perfusion flow through the microdialysis catheter. On the other hand the colloid may also increase the dialysate concentration by preventing a dilution of the surrounding interstitial space. Added dextran reduced loss of perfusion fluid but did not change recovery (Rosdahl, Ungerstedt et al. 1997).

The compound of interest can be added to the perfusion fluid at several different concentrations. Using linear regression it is possible to calculate the point where no diffusion occurs, point of no-net-flux (Lonnroth, Jansson et al. 1987). The interstitial concentration of the compound of interest must be constant and recovery is assumed not to change during the experiment.

Dialysate concentrations can be measured at several flow rates and the interstitial concentration is calculated at zero flow, the zero-flow method. It is then assumed that the dialysate concentration equals the interstitial concentration at zero flow (Ekblom, Gardmark et al. 1992).

Insertion of microdialysis probes has been shown to cause tissue trauma which could influence the results of microdialysis experiments. To allow "tissue equilibration" i.e. to provide time for the initial trauma to subside, probe perfusion for more than half an hour has shown to be sufficient before starting the analysis based on findings of increased thromboxane B₂, glucose and lactate after probe insertion that reach baseline within this time range (Chaurasia, Muller et al. 2007).

Microdialysis is ideal for analysis of low molecular weight analytes that are particularly important to energy metabolism. This set includes, e.g glucose, lactate, glycerol and pyruvate. Other suitable substances for microdialysis includes catecholamines and neurotransmitters (Justice 1993). Larger molecules such as peptides and proteins represent a challenge because of their size and varied chemical and physical properties (Ao and Stenken 2006). Since probes are usually perfused with aqueous solutions the technique is best suited for the study of water-soluble drugs but the technique is also well used in the study of eicosanoids such as prostaglandins with reproducible results (Sun and Stenken 2003).
3.5.3 Metabolites

3.5.3.1 Markers of stress and ischemia – pyruvate, lactate and glucose

Glucose is processed in the glycolytic pathway into two molecules of pyruvate: Glucose (C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}) + 2NAD\textsuperscript{+} + 2ADP + 2P → 2 Pyruvate (CH\textsubscript{3}COCOO\textsuperscript{-1}) + 2NADH + 2H\textsuperscript{+} + 2ATP + 2H\textsubscript{2}O. In an anaerobic environment pyruvate is converted to lactate: pyruvate + NADH + H\textsuperscript{+} → lactate + NAD\textsuperscript{+}. This has a net yield of two molecules of ATP and lactate is accumulated. Glucose can be mobilised from various substrates in the gluconeogenesis, or from glycogen (glycogenolysis). Energy can also be mobilised from triglycerides (lipolysis, beta oxidation). In an aerobic environment the cell can produce much more ATP. Pyruvate and NADH + H\textsuperscript{+} is then further processed in the mitochondria of the cell (pyruvate decarboxylation, citric acid cycle, electron transport chain, oxidative phosphorylation) into approximately 34 additional molecules of ATP for each glucose molecule.

Ischemia of the synovial membrane can be caused by a tourniquet or by distention of the joint capsule during arthroscopic surgery. Tourniquet-induced ischemia resulted in accumulation of lactate and decreased glucose concentrations that were rapidly reversed after the release of the tourniquet (Korth, Merkel et al. 2000; Ostman, Michaelsson et al. 2004). The revascularisation of the tissue can give rise to further damage – the reperfusion paradox (Korthuis and Granger 1993).

The neuroendocrine stress induced by the surgical trauma can induce intraoperative and postoperative hyperglycemia. Glucagon, epinephrine and cortisol are the primary hormones that are secreted in perioperative stress (Akhtar, Barash et al. 2010). Glucose production increases by stimulating gluconeogenesis and glycolytic pathways, whereas glucose clearance decreases due to peripheral insulin resistance (Thorell, Nygren et al. 1999). Insulin resistance and the hyperglycemic response are directly related to the magnitude of surgical trauma (Thorell, Efendic et al. 1993). Regional, spinal or epidural anesthesia with local anesthetics, propofol and opioids seem to be able to blunt such neuroendocrine stress response (Kehlet and Brandt 1979; Jensen, Berthelsen et al. 1980; Sebel, Bovill et al. 1981; Giesecke, Hamberger et al. 1988; Schricker, Carli et al. 2000).

Inhalation anesthetics also have significant effects on the regulation of metabolic processes resulting in alterations of glucose, fatty acids and protein metabolism. High doses or prolonged use may increase glucose concentration (Diltoer and Camu 1988; Iwasaka, Itoh et al. 1996; Lattermann, Schricker et al. 2001).
3.5.3.2 Markers of cell death and tissue injury – glycerol

The most abundant constituent in the cell membrane is phospholipids arranged in a bilayer with a hydrophobic tail and a hydrophilic head (figure 3). This arrangement allows for passive diffusion of hydrophobic molecules but the diffusion of polar solutes is controlled by embedded transmembrane proteins. Due to lack of oxygen and substrates for energy metabolism in tissue trauma and ischemia the cell can no longer maintain the ATP dependent ion gradient over the cell membrane resulting in Ca^{2+} influx and activation of intracellular phospholipases. These lipases will degrade the cell membrane. Phospholipids have either a glycerol molecule or a sphingomyelin as its backbone and free fatty acids and glycerol is an end product of cell membrane degradation. The amount of glycerol released may therefore be a marker of cell death and tissue damage (Marklund, Salci et al. 1997; Hillered, Valtyssoon et al. 1998). Trauma and neuroendocrine stress also affect lipolysis and glycerol release. Surgical trauma increases the lipolytic activity by circulating catecholamines (Fellander, Eleborg et al. 1996).

Figure 3. The cell membrane (wikipedia.org)

3.5.3.3 Markers of inflammation and pain - PGE\textsubscript{2} and Glutamate

Knee joint inflammation causes sensitisation of both peripheral and central nociceptive neurons. Prostaglandin and glutamate contribute to neuronal sensitisation at both sites. There are increased glutamate concentrations in tissues with inflammation or chronic pain (Lawand, McNearney et al. 2000; McNearney, Speegle et al. 2000; Alfredson, Forsgren et al. 2001; Jean, Wen et al. 2005; Schizas, Lian et al. 2009). The excess glutamate in inflamed tissues is likely derived from a variety of sources such as immune cells and direct release from neuronal endings. The increase in glutamate concentration in the knee joint was abrogated by administration of 1 % lidocaine directly into the joint. Lidocaine blocks nerve conduction by decreasing permeability of the membrane to sodium ions during depolarisation. This suggests that sensory neurons in the joint play an important role in the release of glutamate (Lawand, McNearney et al. 2000). Activation of opioid receptors, present on the peripheral endings of small-
diameter afferent fibers, can regulate noxious stimulus induced excitatory amino acid release (Jin, Nishioka et al. 2006).

Glutamate acts on receptors whose major function appears to be modulation of synaptic plasticity. Glutamate receptors are ionotropic or metabotropic. The metabotropic receptors act indirectly on the ionotropic AMPA and NMDA receptors. The interaction between the receptors may induce a "wind-up" mechanism and hyperexcitability (Dickenson, Chapman et al. 1997).

Spinal prostaglandins are involved in the development of inflammation-induced spinal hyperexcitability by interaction of prostaglandins and glutamate AMPA and NMDA receptors, but prostaglandins are not required for the maintenance of central sensitisation (Vasquez, Bar et al. 2001). PGE₂ increases activity of AMPA and NMDA receptors and activation of these receptors in the dorsal horn neurons is thought to be a key mechanism in the induction of central sensitisation (Neugebauer, Lucke et al. 1993). After the initial stimulation of glutamergic synapses endogenous prostaglandins in the spinal cord does not seem to be involved in the maintenance of central sensitisation. Spinal administration of NSAID does not reduce NMDA activity in dorsal horn neurons when inflammation is established (Vasquez, Bar et al. 2001). However, systemic administration of NSAIDs is an effective treatment modality in inflamed joint disease and NSAIDs exert analgesic effects at other sites, such as in the inflamed knee joint (Creamer 2000).

PGE₂ is formed from arachidonic acid through the enzymes cyclooxygenase 1 and 2 (COX 1 and 2). These enzymes, particularly COX 2, is markedly upregulated in the spinal cord in acute and chronic peripheral inflammation (Samad and Abdi 2001). NSAID treatment significantly reduces synovial fluid PGE₂ and down regulate COX-2 mRNA and protein expression at the synovial membrane (Alvarez-Soria, Largo et al. 2006).

**3.5.3.4 Marker of blood flow – Ethanol ratio**

The concentration of metabolites in the interstitial compartment depends not only on local metabolism but also on the delivery and removal of the substances by the microcirculation. It is essential to measure any changes in local blood flow during the experiment. The ethanol escape method has been shown reliable and reproducible. Ethanol is an ideal blood flow marker since it is not locally produced or degraded, does not by itself affect local blood flow and does not influence local carbohydrate or lipid metabolism. The amount of ethanol diffusing from the perfusion fluid to the extracellular compartment increases with increasing blood flow. The ethanol outflow-inflow ratio will therefore be inversely related to blood flow changes, thus increased ethanol ratio reflects decreased blood flow (Hickner, Rosdahl et al. 1991; Fellander, Linde et al. 1996).
4 AIMS OF STUDIES AND HYPOTHESES

The aims of these studies were to

- monitor local synovial changes in classic metabolic compounds and PGE$_2$ in relation to pain experienced by the patient undergoing knee arthroscopy.
- monitor the local changes in classic metabolic compounds and PGE$_2$ with different magnitudes of surgical trauma.
- monitor the effects on local metabolism and inflammation by intervention with intra-articularly administered morphine and ketorolac.
- monitor the effects on local temperature, metabolism and inflammation by application of a combined compression and cooling device (Cryo/Cuff).

Our hypotheses were

- degree of pain as experienced by the patient will be reflected by disturbances in synovial metabolism and inflammatory reactions.
- increased magnitude of surgical trauma will be reflected by increased disturbances in synovial metabolism and inflammatory reactions.
- intervention with intra-articular administration of morphine or ketorolac will result in decreased disturbances in local synovial metabolism and inflammatory reactions.
- intervention with local application of a combined cooling and compression device postoperatively will result in lower knee joint temperatures and decreased disturbances in local synovial metabolism and inflammatory reactions.
5 MATERIAL AND METHODS

The studies were approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Study 3 was also approved by the Swedish Medical Products Agency.

5.1 PATIENTS

All patients received standardised information and written informed consent was given and approved before inclusion.

In total 130 patients completed the studies; study 1 14 patients, study 2 30 patients, study 3 60 patients and study 4 40 patients. In study 2 14 patients from study 1 were included (figure 4).

Inclusion criteria were indication for arthroscopy due to clinical signs of meniscus injury with positive McMurray and/or Apleys test and adequate subjective inconvenience (Study 1-4) or a symptomatic ACL injury requiring reconstruction (Study 2). The ACL injury was diagnosed clinically with positive Lachmann and pivot shift tests. ACL patients had a presurgery magnetic resonance imaging scan or diagnostic arthroscopy.

Exclusion criteria were systemic inflammatory diseases, use of anti-inflammatory medication preoperatively, or previous diagnosis of osteoarthritis. In study 3 patients with intolerance and/or allergy to non-steroidal anti-inflammatory drugs or morphine were excluded. Five patients in study 3 and 4 patients in study 4 were excluded due to technical problems with the microdialysis catheter. The number of patients excluded due to technical problems was not registered in study 1 and 2.

Randomisation was performed in study 3 and 4 before surgery by preparing sealed envelopes, one for each patient, containing the planned protocol. The envelopes for the patients where we experienced technical problems with the microdialysis procedure were replaced. After exclusion due to technical problems 60 patients in study 3 and 40 patients in study 4 were randomised into equal subgroups (figure 4).
Figure 4. Patient flow chart.
5.2 MICRODIALYSIS

The principles of microdialysis have been described in detail above. A microdialysis catheter (probe) with a pore size of 20 kDa (CMA 60; CMA Microdialysis AB, Solna, Sweden) was implanted in the synovial membrane under arthroscopic visual control on the medial side of the knee (see pictures on cover page). As a reference a similar catheter was inserted into the subcutaneous adipose tissue of the contralateral thigh. The probe consists of a thin, double lumen, concentric plastic tube with a 30 mm semipermeable tubular membrane at its distal end. A physiologically compatible perfusion fluid (Perfusion fluid T1, CMA Microdialysis AB, Solna, Sweden) is pumped through the outer tube in the catheter with a pump (CMA 106, CMA Microdialysis AB, Solna, Sweden) and flows underneath the semi-permeable membrane where the exchange between the interstitial fluid and the perfusion fluid takes place. At the tip of catheter the fluid enters a hole in the inner tube and is finally collected in a vial (Microvial, CMA Microdialysis AB, Solna, Sweden) (figure 5 a, b). The perfusion speed was set at 2.0 µL/min and the system was equilibrated 40 minutes before samples were collected. In study 1 and 2 10 samples (fractions) were collected every 20 minutes and in study 3 and 4 6 samples (fractions) were collected every 40 minutes. Thus, sample 1 (fraction 1) monitored the metabolic status during 40 to 60 or 40 to 80 minutes after completion of surgery.

Figure 5 a, b. The principles of the microdialysis catheter (a) and the CMA 60 microdialysis catheter (b) (CMA Microdialysis).
5.3 PROCEDURE

General anesthesia was induced and maintained with propofol, alfentanil and sevoflurane as needed. All patients received standardised premedication (2000 mg paracetamol/60 mg codeine) and all patients received 20 ml of bupivacain with adrenaline (5 mg/ml + 5µg/ml) injected intra-articularly after completion of surgery. The joint was irrigated with glucose-free saline (NaCl 9 mg/ml). A tourniquet was used only in the patients that underwent arthroscopically assisted ACL reconstruction (study 2).

In study 3 the nurse anesthetist prepared the medication (1 ml morphine 10 mg/ml in 9 ml of NaCl 9 mg/ml, 2 ml ketorolac 30 mg/ml in 8 ml of NaCl 9 mg/ml or 10 ml NaCl) that was injected intra-articularly by the surgeon approximately 10 min before start of the surgical procedure in a double-blinded fashion.

In study 4 a cooling and compression device (Cryo/Cuff, Aircast, USA) was applied on the operated knee in patients randomised to intervention.

5.3.1 Metabolites, PGE₂ and ethanol ratio

The metabolites glucose, lactate, glycerol (Study 1-4), pyruvate (Study 1) and glutamate (Study 4) were analysed with a CMA 600 microdialysis analyzer using an enzymatic fluorometric method (CMA Microdialysis, Solna, Sweden).

In all patients, tissue blood flow was indirectly monitored by adding 50 mmol/l of ethanol to the dialysate solvent (Study 1-4). The ethanol escape ratio, described in detail above, was calculated. Ethanol was analysed with an enzymatic spectrophotometric method.

PGE₂ was analyzed with ELISA (prostaglandin E₂, EIA Kit-Monoclonal, Cayman Chemical Company, Ann Arbor, MI, USA)(Study 1-4).
5.3.2 Temperature

Temperatures were recorded with specific temperature probes on four local and one central (core body) location: intraarticular, in the joint capsule, on the skin of the operated knee and on the skin of the contralateral knee (joint capsule MTS-40015, intraarticular ER400-9, skin STS-400, all probes from Smiths Medical, Rockland, MA, USA). For core body temperature an ear probe was used (Braun Thermoscan, Type 6022, Germany). The probes were connected to a calibrated monitor Datex-Ohmeda S/5 (Datex-Ohmeda, Helsinki, Finland)(Study 4).

5.3.3 VAS, NRS, Rescue Medication

Postoperative pain was evaluated with the visual analogue scale (VAS), 0=no pain, 10= worst imaginable pain (Study 3-4) and requirements of postoperative analgesics of the opioid ketobemidone (Study 1-4). The nurse responsible for postoperative surveillance was instructed to offer 2-5 mg of ketobemidon if the patient experienced pain with VAS more than or equal to 4.

Upon discharge, the patients received a pain questionnaire estimating the average level of pain experience during activity and rest for the last 24 hours using numeric rating scale (NRS, 0= no pain, 10= worst imaginable pain)(Study 3-4) in the evening once daily the first 7 days, after 14 days and 30 days after surgery.
5.4 STATISTICS

Data were analysed using Statistica 8.0 (StatSoft Inc, Tulsa, OK, USA) and the procedure Mixed in SAS® (SAS® System 8.2 and 9.1, SAS Institute Inc., Cary, NC, USA). To compare patient demographic data Student t-test, the Mann-Whitney U test or Chi-square test were done according to the type of distribution using Sigmastat 3.0 (SPSS, Chicago, IL). P<0.05 was considered statistically significant.

First we computed a Mixed model with Condition (knee and fat) and Time (Study 1-2: ten time points, fractions Study 3-4: six time points, fractions) as the within-subjects variables and Group (Study 1: Opioid, No Opioid; Study 2: Arthroscopy, ACL; Study 3: Morphine, Ketorolac, Placebo; Study 4: NO Cryo/Cuff, Cryo/Cuff) as the between-subjects variable. Because the covariance structures with two within-subjects factors are limited and our main hypothesis called for interactions of Group with Time, we performed separate Mixed models by condition. (Littell et al. 1996). For these models a first order autoregressive covariance structure was considered most appropriate. Because the variable Time is quantitative, we also model the response variables as a polynomial function of Time. This gives smoothed trends over Time. The most important question was whether the two groups differed regarding increasing or decreasing trends over time. Therefore, first- and second order model was sufficient. The data for some of the variables have been log-transformed in order to meet the requirements for an adequate Mixed model.

VAS over fractions and NRS over days were analysed using Kruskal-Wallis ANOVA ranks or Mann-Whitney U test according to the type of distribution in comparison between groups. The effects within each group were analyzed using Friedman ANOVA by ranks, followed by multiple comparisons between days and fractions (Study 3-4).

6 RESULTS

6.1 PATIENTS

Demographic variables in the groups were similar in all studies but study 1 where age distribution differed between the groups (table 1). No adverse effects such as nausea or vomiting were caused by intra-articular administration of morphine or ketorolac. No adverse effects such as cold injuries were seen by the application of a cooling and compression device. No patients reported discomfort using the cooling and compression device.
TABLE 1. Patient Demographics

<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th>Male/Female</th>
<th>Age, years</th>
<th>Body Mass Index</th>
<th>Operating Time, min</th>
<th>Rescue Medication, Ketobemidon</th>
<th>Surgery/diagnostic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Opioid</td>
<td>4/2</td>
<td>29.8 (8.8)</td>
<td>27.8 (4.5)</td>
<td>47 (13.1)</td>
<td>5/1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No opioid</td>
<td>6/2</td>
<td>40.3 (6.6)</td>
<td>28.0 (3.4)</td>
<td>38.6 (12.7)</td>
<td>5/3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>NS¹</td>
<td></td>
<td>NS²</td>
<td>NS²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Arthroscopy</td>
<td>13/7</td>
<td>34.6 (9.9)</td>
<td>26.6 (4.4)</td>
<td>37.9 (14.4)</td>
<td>8/20</td>
<td>14/6</td>
</tr>
<tr>
<td></td>
<td>ACL</td>
<td>6/4</td>
<td>28.9 (9.5)</td>
<td>24.6 (3.2)</td>
<td>111.2 (24.8)</td>
<td>6/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>NS¹</td>
<td></td>
<td>NS²</td>
<td>NS²</td>
<td>P&lt;0.001²</td>
<td>NS³</td>
</tr>
<tr>
<td>3</td>
<td>Morphine</td>
<td>11/9</td>
<td>37.7 (14.5)</td>
<td>24 (6.8)</td>
<td>29.7 (16.6)</td>
<td>5/15</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Ketorolac</td>
<td>13/7</td>
<td>40.7 (10.3)</td>
<td>25 (7.1)</td>
<td>27.4 (9.7)</td>
<td>5/14</td>
<td>15/5</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>10/10</td>
<td>37.3 (13.9)</td>
<td>23.3 (9.1)</td>
<td>32 (15.9)</td>
<td>6/12</td>
<td>14/6</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>NS³</td>
<td></td>
<td>NS⁴</td>
<td>NS⁴</td>
<td>NS²†</td>
<td>NS³</td>
</tr>
<tr>
<td>4</td>
<td>NO cooling and compression</td>
<td>10/10</td>
<td>36.4 (10.0)</td>
<td>28.1 (3.4)</td>
<td>31.3 (13.5)</td>
<td>8/12</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>Cooling and compression</td>
<td>12/8</td>
<td>36.9 (10.0)</td>
<td>24.5 (9.4)</td>
<td>35.9 (17.1)</td>
<td>5/15</td>
<td>12/8</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>NS³</td>
<td></td>
<td>NS²</td>
<td>NS⁵§</td>
<td>NS²</td>
<td>NS³</td>
</tr>
</tbody>
</table>

Data is presented as Mean and SD, Fisher exact test¹, Student t test², Chi-square test³, One-way ANOVA test⁴, Mann-Whitney U test⁵. NS indicates not significant.

Missing data for one patient in the morphine group, one patient in the ketorolac group, and two patients in the placebo group. † No data available for one patient in the ketorolac group and two patients in the placebo group. § Missing data for two patients in the cooling and compression group.
6.2 PGE₂, METABOLITES AND ETHANOL RATIO

6.2.1 PGE₂

The changes in the general postoperative course of PGE₂ were reproduced in all studies for the arthroscopy groups. The concentration of PGE₂ in the reference tissue was low or not detectable in all studies.

PGE₂ levels were higher initially and slowly decreased over time with more postoperative pain as shown by requirements of rescue medication, ketobemidon (study 1). The levels of PGE₂ decreased over time in the group requiring postoperative ketobemidon (Opioid) (p=0.0077), while the group with no requirements of postoperative pain relief (No Opioid) showed stable levels over time (figure 6).

![Synovium Graph]

Figure 6. PGE₂ in synovium, study 1

Dialysate levels of PGE₂ (mean and SD) over 3 h after knee arthroscopy. There was a significant trend toward decreased levels of PGE₂ over time in the synovial membrane of the patients receiving opioids (p=0.0077), but not among those who did not require additional opioids.
PGE$_2$ levels were lower or not detectable after arthroscopically assisted reconstruction of the anterior cruciate ligament (ACL) (figure 7) (table 2).

Synovium

![Graph showing PGE$_2$ levels in synovium](image)

Figure 7. PGE$_2$ in synovium, study 2.

Prostaglandin E$_2$ levels were detected more frequently in the arthroscopy group (table 2) and within a higher magnitude compared to the ACL group. Data presented as mean and SD.
TABLE 2. PGE2 in synovial membranes of arthroscopy and ACL groups, study 3

<table>
<thead>
<tr>
<th></th>
<th>Arthroscopy (n=14; 140 measurements)</th>
<th>ACL (n=6; 60 measurements)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Synovium</td>
<td>Reference</td>
</tr>
<tr>
<td>Detectable</td>
<td>139</td>
<td>91</td>
</tr>
<tr>
<td>Non-detectable</td>
<td>1</td>
<td>49</td>
</tr>
</tbody>
</table>

Most of the values of PGE2 were below detection limits in the synovial membrane of the ACL group and in the reference tissue of both groups compared to the synovial membrane in the arthroscopy group. There was a significant difference in the proportion of detectable values. Arthroscopy synovium vs ACL synovium (P < 0.001); arthroscopy reference vs ACL reference (P < 0.001); arthroscopy synovium vs arthroscopy reference (P < 0.001); and ACL synovium vs ACL reference (P = 0.029) using the chi-square test.

Intra-articular administration of morphine or placebo (NaCl) did not change the postoperative course of PGE2 compared to our previous findings in the arthroscopy groups. Intra-articular administration of ketorolac on the other hand lowered the PGE2 levels both in synovium and reference. This difference between the groups was significant (synovium p<0.0001; reference p<0.0001) (figure 8).

Application of a combined compression and cooling device did not induce a difference in PGE2 concentrations. In the group that received a cooling and compression device there was large individual differences in how much the temperature was lowered but a positive correlation with the temperature in the joint capsule; higher temperatures resulted in higher PGE2 levels, were found (table 3).

TABLE 3. Correlation for PGE2, lactate and ethanol ratio with temperature in joint capsule

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Fraction 1</th>
<th>Fraction 2</th>
<th>Fraction 3</th>
<th>Fraction 4</th>
<th>Fraction 5</th>
<th>Fraction 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE2</td>
<td>Cooling and compression</td>
<td>p=ns</td>
<td>p=0.002</td>
<td>p=0.007</td>
<td>p=0.002</td>
<td>p=0.0015</td>
<td>p=0.001</td>
</tr>
<tr>
<td>Lactate</td>
<td>Cooling and compression</td>
<td>p=0.022</td>
<td>p=(0.074)</td>
<td>p=(0.089)</td>
<td>p=(0.075)</td>
<td>p=0.042</td>
<td>p=(0.069)</td>
</tr>
<tr>
<td>Ethanol ratio</td>
<td>All groups</td>
<td>p=ns</td>
<td>p=ns</td>
<td>p=(0.089)</td>
<td>p=0.016</td>
<td>p=0.008</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

There was a positive correlation with temperature for PGE2 in fraction 2-6 and for lactate in fraction 1 and 5 in the joint capsule for the cooling and compression group. Higher temperature resulted in higher PGE2 and lactate values. There was a positive correlation with temperature for ethanol ratio in the joint capsule when all groups were summarised in fraction 4-6.
There was a significant difference in overall levels of PGE2 (P = 0.0001) and change over time (P = 0.0246) between synovium and reference tissue. The synovial tissue showed a significant difference in the PGE2 levels between the treatment groups (P<0.0001). The ketorolac group showed low levels of PGE2. The reference tissue also showed significance in levels between the groups (P <0.0001) with lower levels in the ketorolac group. Data are presented as mean and SD.

Figure 8. PGE2 in synovium and reference, study 3.
6.2.2 LACTATE

The postoperative changes in lactate levels were similar in the arthroscopy groups of all studies without intervention with intra-articular medication or cooling and compression. Lactate showed high and increasing levels over time in the synovial tissue compared to the reference tissue.

There were no significant differences in lactate concentrations in relation to requirements of postoperative rescue medicine in neither the synovial or reference tissue (Study 1).

The increasing lactate change over time that was seen in the arthroscopy groups without intervention were not repeated in the ACL reconstruction group. In study 2 the arthroscopy group showed increasing lactate with time (p<0.0001) while the ACL groups showed stable levels. This difference in course over time was statistically different (p=0.0221) [figure 9].

---

Figure 9. Lactate in synovium, study 2.

There was a significantly increased level of lactate in the synovial membrane after minor surgery (arthroscopy group; P < .0001) compared to stable levels after major surgery (anterior cruciate ligament reconstruction group; P = NS). This difference in course over time was significant (p=0.0221).
The levels or change over time for lactate were not changed when intervening with intra-articular administration of morphine, ketorolac or placebo (Study 3).

With application of cooling and compression the previously shown increase of lactate over time was absent. The group with no cooling and compression showed an increase over time ($p<0.0001$) but not the cooling and compression group. This difference in change over time between synovial tissue in the two treatment groups was significant ($p=0.0018$) (figure 10).

A correlation between lactate and the joint capsule temperature was indicated in the cooling and compression group (table 2). Higher temperature results in higher lactate values.

![Figure 10. Lactate in synovium, study 4.](image)

An increase over time in the synovium was seen in the NO cooling and compression group ($p<0.0001$) but not in the cooling and compression group ($p=0.2049$) compared to reference tissue. There was also a significant difference regarding change over time between synovial tissue in the two treatment groups ($p=0.0018$).
6.2.3 GLUCOSE

The postoperative changes in glucose levels were similar in the arthroscopy groups of all studies regardless of intervention with intra-articular medication or cooling and compression. Glucose showed decreasing levels over time in the synovial tissue compared to the reference tissue.

If separating the patients with less postoperative pain, i.e. not requiring rescue medication, the postoperative course of glucose was stable. In the group requiring rescue medication we observed decreasing levels as found previously (p<0.0001). This difference in the postoperative levels over time was statistically significant (p=0.0207) (Study 1) (figure 11).

![Synovium](image)

Figure 11. Glucose in synovium, study 1.

In the groups requiring additional rescue medication due to postoperative pain decreasing levels of glucose over time were found (p<0.0001). The levels in the group not requiring rescue medication were stable. This difference between the groups in postoperative glucose levels over time was significant (p=0.0207).
The decreasing glucose changes over time that was seen in the arthroscopy groups were not replicated in the ACL reconstruction group. In study 2 the arthroscopy group showed decreasing glucose with time (p=0.005) while the ACL group showed stable levels. Significantly higher levels of glucose in the synovial membrane in the ACL group were also found (p=0.0273) (figure 12).

Synovium

![Graph showing glucose levels over fractions for ACL and Arthroscopy groups.](image)

Figure 12. Glucose in synovium, study 2.

There was a significant consumption of glucose in the synovial membrane after minor surgery (arthroscopy group; P = .005) compared to stable levels after major surgery (anterior cruciate ligament reconstruction group; P = NS). The absolute glucose levels in the synovial membrane of the anterior cruciate ligament reconstruction group were also significantly higher than in the arthroscopy group (P = .0273).
6.2.4 GLUTAMATE

Glutamate was studied in study 3 and 4. In general higher (Study 3: p=0.0007; Study 4: p=0.0007) and decreasing (Study 3: p<0.0001; Study 4: p=0.050) levels over time were found in synovial tissue compared to reference tissue.

Figure 13. Glutamate, study 3.

There were significantly higher levels of glutamate (P = 0.0007) that decreased over time (P < 0.0001) in synovial tissue compared with reference tissue. There was a numerical decrease in all 3 groups; however, it reached statistical significance only in the morphine (P = 0.0049) and ketorolac (P < 0.0021) groups. There were no differences between the groups regarding levels or change over time in reference tissue. Data are presented as mean and SD.
Intervention with intra-articular administration of morphine, ketorolac or placebo resulted in a numerical decrease of glutamate concentration over time in all three groups but it reached significance only in the morphine (p=0.0049) and ketorolac (P<0.0021) groups (Study 3). This difference in course over time was not significant between the groups (figure 13). Application of cooling and compression did not influence glutamate concentrations.

6.2.5 GLYCEROL

Magnitude of subjective postoperative pain (study 1) or a supposedly greater surgical trauma (study 2) did not impact postoperative glycerol levels. Nor did application of a cooling and compression device influence postoperative glycerol concentrations (study 4). No differences between the groups or synovia and reference regarding levels or change over time could be seen in study 1, 2 and 4.

With intra-articular administration of ketorolac, morphine and placebo, for the first time a general difference in change over time between synovium and reference with increasing glycerol concentrations over time in the synovium was demonstrated (p<0.0001). This change over time was only significant for the ketorolac group (p=0.0078)(figure 14).

Figure 14. Glycerol, study 3.

There were no differences in levels between synovium and reference tissue, but a significant difference in change over time could be found (P < 0.0001). An increasing change over time was significant only for the ketorolac group in the synovial membrane (P = 0.0078). No significant differences between the groups in the reference tissue could be found. Data are presented as mean and SD.
6.2.6 PYRUVATE

Pyruvate was studied in study 1. There were no differences in pyruvate concentrations in relation to requirements of additional postoperative analgesia (study 1).

6.2.7 ETHANOL RATIO

Blood flow measured as ethanol ratio was stable in the knee arthroscopy groups without postoperative application of Cryo/Cuff (Study 1, 2 and 3). Blood flow was also stable in the ACL group and no difference in levels or change over time could be seen between the ACL group and arthroscopy group (Study 2).

With intra-articular administration of Ketorolac the absolute levels for ethanol were increased and resulted in significant difference in levels compared to the groups with intra-articular administration of morphine or placebo (p=0.0102) but no difference in changes over time were seen indicating stable blood flow. It should be remembered that the commercially available ketorolac preparation used in this study contains ethanol.

With application of cooling and compression the ethanol ratio increased significantly over time in synovium compared to the group not receiving cooling and compression (p=0.0484) (figure 15). Ethanol ratio was correlated to the temperature in the joint capsule in fraction 4-6 (table 3).
The ethanol ratio increased significantly over time in the synovium of the cooling and compression group compared to synovium in the NO cooling and compression group (p=0.0484). This indicates lower blood flow in the cooling and compression group.

### 6.2.8 VAS, NRS AND RESCUE MEDICATION

VAS and NRS were studied in study 3 and 4. No difference in postoperative pain scoring with VAS, the first 4 hours, was seen with intra-articular morphine, ketorolac or placebo treatment (study 3). On the other hand, the course of decreasing NRS, the first 14 days post-surgery, was significantly different between the groups (p=0.0065) (figure 15). The ketorolac group had a lower baseline value of NRS and, consequently, did not have the same decrease in levels of NRS over time. This change over time was significantly different from the course of the morphine (p=0.0025) and placebo (p=0.0007) groups. Application of cooling and compression did not influence VAS 4 hours postoperatively or NRS the first 14 days after surgery (study 4). Day 30 was excluded due to too many missing values in both study 3 and 4.
Additional requirements of rescue medications (ketobemidon) were studied in study 1-4 (table 1). In study 1 the study was designed to study differences in local postoperative markers in relation to the need for additional rescue medication. In study 2 no difference in the need for rescue medication due to postoperative pain was seen when comparing a minor surgery, knee arthroscopy, with a supposed major surgery, arthroscopically assisted ACL reconstruction. Intra-articular administration of morphine, ketorolac or placebo did not influence postoperative need for rescue medication (study 3) neither did application of cooling and compression (study 4)(table 1).

Figure 16. NRS the first 14 days, study 3.

NRS during 14 days after surgery. All 3 groups showed decreasing levels of pain intensity (NRS) over time, but the course of this decrease was significantly different between the groups (P = 0.0065). Data are presented as mean and SD.
6.2.9 TEMPERATURE

The temperature was lower in the cooling and compression group at all three measured locations on the operated knee (skin $p<0.001$, joint capsule $p<0.001$ and intra-articular $p=0.0002$). A significant difference in change over time was also seen (skin $p<0.0001$, joint capsule $p<0.001$ and intra-articular $p<0.001$) (figure 17). The ear temperature and contralateral knee joint skin temperature was stable and no differences between the groups were observed.

![Temperature graph]

Figure 17. Temperature, study 4.
7 DISCUSSION

7.1 GENERAL POSTOPERATIVE EFFECTS

A hypermetabolic state with an increase in carbohydrate consumption, shown by a decrease in glucose and increase in lactate concentrations signaling anaerobic metabolism, has been shown postoperatively after knee arthroscopy (Felländer-Tsai et al. 2002). In these studies of knee arthroscopy the changes in postoperative synovial metabolism were repeated. These findings are similar to findings of glucose and lactate in skeletal muscle during 90 min of tourniquet-induced ischemia, but the concentrations were rapidly normalised after the release of the tourniquet (Ostman, Michaelsson et al. 2004). Knee arthroscopy in our studies was performed without a tourniquet but the irrigation fluid used during arthroscopy cause knee joint distention and probably a relative ischemia. In these studies the changes of glucose and lactate concentrations were increasing the first four postoperative hours indicating a difference in response to ischemia for synovial tissue compared to skeletal muscle. A tendency towards normalisation was not seen the first four postoperative hours. It would be of great interest to further study the effects on synovial metabolism after knee arthroscopy beyond the four hours studied. The prolonged ischemic reaction in synovial tissue could implicate a more sensitive reaction to ischemia in synovial tissue. It is possible that arthroscopy with low pressure irrigation fluid should be endeavoured.

The synovium can be regarded as a metabolically active organ producing synovial fluid containing lubricants and nutrients for the joint. The nutrition of the avascular cartilage depends greatly on the synovial membrane and resulting synovial fluid. An impaired function may induce deleterious effects on joint tissues. An impaired function of synovial tissue is seen in OA and after joint trauma (Elsaid, Jay et al. 2005; Elsaid, Fleming et al. 2008). The long-term consequences of the surgical trauma to the knee joint after knee arthroscopy are unknown. In general no apparent effects on glycerol concentration were seen in these studies. This could indicate preserved cell membrane integrity and perhaps no permanent cell injury since glycerol is an abundant constituent in the cell membrane (Marklund, Salci et al. 1997; Hillered, Valtysson et al. 1998). This could also indicate that there is no profound reperfusion injury after knee arthroscopy (Korthuis and Granger 1993).

Knee arthroscopy in the present setting did not seem to induce a profound postoperative neuroendocrine stress response. The combined action of glucagon, catecholamines, cortisol and increased peripheral insulin resistance would have resulted in hyperglycemia (Thorell, Nygren et al. 1999; Akhtar, Barash et al. 2010). Possibly the use of intra-articular local anesthetics blunted the local effects of the neuroendocrine response (Kehlet and Brandt 1979) or the surgical trauma was not of sufficient magnitude to elicit local effects. However, the high initial glucose and PGE₂ concentrations the first fraction could also reflect an intraoperative stress response with hyperglycemia and inflammation that was normalised during the first four postoperative hours. The glucose is then utilized in the tissue in anaerobic metabolic pathways resulting in accumulation of lactate.
Levels of PGE2 and glutamate were generally high and decreasing throughout the postoperative period. This is in agreement with the findings for PGE2 in painful and inflammatory joint disease (Egg 1984; Lawand, McNearney et al. 2000; McNearney, Speegle et al. 2000; Trumble, Billinghurst et al. 2004). An important interaction between PGE2 and glutamate exists (Vasquez, Bar et al. 2001).

7.2 METABOLIC EFFECTS OF PAIN

Management of postoperative pain is of great importance in order to allow early mobilisation and ambulatory surgery. Pain is the most important symptom for the individual. Symptomatic pain seems to be only weakly related to the surgical trauma in knee arthroscopy and also weakly related to the arthroscopic findings of knee pathology (study 1 and 2). A better understanding and knowledge of the pathogenesis of joint pain is necessary to be able to optimise pre-, intra- and postoperative management in knee joint arthroscopy.

The findings in study 1 indicated that local tissue changes in metabolic and inflammatory compounds measured by microdialysis in the synovial membrane postoperatively may be correlated to the magnitude of pain as experienced by the patient. The general metabolic and inflammatory findings were high and decreasing PGE2 and glucose postoperatively. If separating the patients requiring additional postoperative analgesia, i.e, rescue medication with ketobemidon, from those without profound postoperative pain the latter group did not show the same inflammatory and metabolic reaction. In this group PGE2 and glucose levels were stable over time.

These results may be explained by a more pronounced surgically induced stress reaction in those experiencing more postoperative pain. The group that required additional postoperative rescue medication had higher concentrations of glucose the first postoperative fractions. This could be a result of increased intraoperative stress which results in neuroendocrine responses and hyperglycemia (Akhtar, Barash et al. 2010). It would be of interest to study synovial metabolism and inflammation intraoperatively but that is a challenge due to the fragile microdialysis catheters available.

The increased concentration of PGE2 in the group experiencing more postoperative pain is in accordance with previous findings for prostaglandins in inflammation and pain (Trumble, Billinghurst et al. 2004) and a good rationale for the use of NSAIDs (Creamer 2000).

The postoperative course of lactate, pyruvate and glycerol were not influenced by requirements of additional postoperative analgesia treatment. This indicated that these metabolites are weaker indicators of pain.

The change in postoperative inflammation and metabolism in study 1 did not seem to be related to the surgical trauma itself since the groups did not differ in that respect. It probably reflects interindividual reactions in the metabolic and inflammatory systems. With better knowledge of the individual differences
in reaction to trauma it would be possible to individualise the surgical pre-, intra- and postoperative management for best result.

7.3 EFFECTS OF INTERVENTION WITH INTRA-ARTICULAR MEDICATION

PGE₂ levels were effectively lowered by ketorolac in synovial tissue but also in the contralateral fat indicating a systemic effect (study 3). No effects on PGE₂ were seen for morphine or placebo. Any local or systemic effects of ketorolac in the operated knee could not be separated since plasma levels of ketorolac were not measured. This finding is in agreement with previously known actions of NSAIDs. NSAIDs is an effective treatment modality in OA and inflamed joint disease and its primary action is by inhibiting prostaglandin synthesis (Creamer 2000).

It is hard to draw any conclusion on the effects of ketorolac and morphine on glutamate. Generally for glutamate there was a high and over time decreasing concentration compared to reference tissue indicating a trauma effect on glutamate. This decrease over time was however significant only in the ketorolac and morphine groups but not in the placebo group. The difference between the groups was on the other hand not significant. Glutamate is a neurotransmitter important for pain sensitisation and is abundant in the central nervous system but also found in peripheral tissues experiencing pain or inflammation (Lawand, McNearney et al. 2000; McNearney, Speegle et al. 2000; Alfredson, Forsgren et al. 2001; Jean, Wen et al. 2005). Glutamate has been suggested as a target for pain medication. Intra-articular effects of ketamine, a glutamate NMDA receptor antagonist, has been studied with contradictory results (Zhang, Min et al. 2004; Castrillon, Cairns et al. 2007; Ayesh, Jensen et al. 2008; Castrillon, Cairns et al. 2008; Ayoglu, Altunkaya et al. 2010). This could be due to involvement of non-NMDA receptors in the pain mechanism. The role for peripheral glutamate in pain after knee arthroscopy should be further studied.

We have shown a correlation between PGE₂ and pain (study 1) but we could not detect any differences in subjective pain scoring the first four postoperative hours by administration of ketorolac intra-articularly. However, the course of NRS the first 14 days after surgery was different in the ketorolac group, in comparison with the morphine and placebo groups, with a stable low NRS over time. Intra-articular ketorolac is used clinically in knee arthroscopy and there are studies supporting its effects (Calmet, Esteve et al. 2004). It is hard to draw any conclusions regarding its effect on subjective pain in this material. However, this was not the primary endpoint in the study design and the study was underpowered for detecting differences in subjective pain scoring.

It should be remembered that NSAIDs may have chondrotoxic effects. The effects of the different commercially available NSAIDs are complex but hypothetically selective COX-2 inhibitors could be favourable (Ding 2002; Chang, Wu et al. 2006; Jean, Wen et al. 2007). We could see a different postoperative course of glycerol in the ketorolac group with increasing glycerol over time. This could indicate an adverse effect of ketorolac. The clinical significance of these findings should be further evaluated before using ketorolac extensively intra-articularly.

An anti-inflammatory effect has been proposed for morphine (Walker 2003). The lack of effects for intra-
articulary administration of morphine in our study could be due to the absence of profound inflammation that may be a prerequisite for peripheral opioid analgesia (Mousa, Zhang et al. 2001; Zollner, Shaqur et al. 2003).

7.4 EFFECTS OF TEMPERATURE

Postoperative application of a combined cooling and compression device was able to maintain a low and stable temperature in the knee joint. Our study showed that application of cooling and compression after knee arthroscopy significantly lowered and maintained a low knee temperature postoperatively by varying degrees with the lowest temperature on skin and diminishing effects in joint capsule and intraarticularly (study 4). Cryotherapy is commonly used for analgesia and enhanced recovery after knee arthroscopy. Cryotherapy is probably a safe procedure, fairly inexpensive and with a high level of patient satisfaction. Its effects on postoperative pain and any effects on recovery and clinical function is however uncertain (Schroder and Passler 1994; Edwards, Rimmer et al. 1996; Dervin, Taylor et al. 1998; Ohkoshi, Ohkoshi et al. 1999; Raynor, Pietrobon et al. 2005). Study 4 was not able to demonstrate differences in postoperative VAS, NRS or use of additional rescue medication due to too low power for subjective pain variables in the study design.

Interestingly a correlation with synovial PGE2 and temperature was shown. A temperature sensitive action in the release of PGE2 has been shown previously (Wang, Xuan et al. 1993; Diestel, Roessler et al. 2008) but to our knowledge this is shown for the first time in vivo in man. These findings may be explained by the reduced actions of the enzymes phospholipase C and A2 crucial for prostaglandin biosynthesis in hypothermia (Majumdar, Gowda et al. 1995). PGE2 concentrations were correlated to the temperature in the joint capsule in the cooling and compression group, but there was no difference in PGE2 levels between the groups cooling and compression and NO cooling and compression probably due to a great individual variation in both temperature and PGE2. Yet, this indicated a positive anti-inflammatory effect with cooling and compression postoperatively.

A correlation between lactate and the joint capsule temperature was also indicated in the cooling and compression group. The previously shown general effects of increasing levels of lactate postoperatively were diminished in the cooling and compression group. This could be a sign of decreased metabolism and probably decreased energy needs of the cells. A cell with high energy demands in an energy deprived surrounding will ultimately succumb. The decreased energy needs of the cells may induce a tissue protective effect in ischemic tissue and mild hypothermia may even decrease the harmful effects of reperfusion in ischemic tissue (Francel, Vander Kolk et al. 1992; Mowlavi, Neumeister et al. 2003).

Application of cooling and compression also imposes changes to the microcirculation (Knobloch, Kraemer et al. 2006). An increase in ethanol ratio in the cooling and compression group was seen indicating lower blood flow. A correlation between ethanol ratio with joint capsule temperature was also indicated. It is not possible to distinguish the effects of hypothermia per se or the decreased blood flow on local metabolism.
Hypothermia reduces cellular metabolism and the metabolic pathways are slowed down. A decrease in the glycolytic pathway would theoretically induce local hyperglycemia (Kelleher, Nauth et al. 1998). On the other hand the decrease in blood flow reduces the availability of blood glucose but also diminishes the possibility for transporting away waste products, as lactate. The effects are complex and somewhat counteracting.

Knee arthroscopy with room tempered irrigation fluid lowers knee temperature per se (Zaffagnini, Allen et al. 1996). In the future it would be interesting to study the effects of cooling of the irrigation fluid used in knee arthroscopy.

7.5 EFFECTS OF INCREASING THE MAGNITUDE OF TRAUMA

Study 2 failed to demonstrate an increase in inflammatory markers or signs of metabolism when increasing the magnitude of the surgical trauma; knee arthroscopy compared to arthroscopically assisted ACL reconstruction. There are however several pre-, intra- and postoperative differences between the groups. The ACL group had morphine administered intra-articularly preoperatively, a tourniquet was used and a cooling and compression device was applied postoperatively. In the ACL group the expression of inflammatory markers and rate of local metabolism were however even lower than in the arthroscopy group. The separate effects of morphine and cooling and compression were further studied (study 3 and 4). No apparent effects of morphine explaining the results seen in study 2 were found. Applying cooling and compression on the other hand imposed effects on both PGE₂ and lactate. In conclusion, the application of cooling and compression likely was responsible for most of the surprising findings in study 2.

Arthroscopically assisted ACL reconstruction surgery is apart from the use of tourniquet and longer operating time also a procedure including drilling of tunnels in the femur and tibia. This makes it possible for the irrigation fluid to escape and possibly resulting in less knee joint distention and intra-articular pressure. A counteracting mechanism to the supposed negative local effects of the tourniquet is possible.

It must also be remembered that an ACL injury per se with any associated cartilage and meniscus injuries may induce other effects on the knee than a degenerative meniscus injury as often seen in the knee arthroscopy group.
8 CONCLUSION

For the first time local metabolic and inflammatory effects of intra-articular administration of ketorolac or morphine and postoperative application of cooling and compression are demonstrated.

The local effect of ketorolac on PGE₂ indicated an effect on inflammation and possibly on nociception, but it should be remembered that 60 mg of ketorolac administered intra-articularly exerts a pronounced systemic effect. The safety of ketorolac and NSAIDs regarding their toxicity on cartilage must be further evaluated.

For morphine no apparent effect on inflammatory markers, metabolism or nociception was found. Intra-articular morphine is commonly used in knee arthroscopy for analgesia but the results on its analgesic effect is contradictory. Intra-articular administration of morphine can be regarded safe since no adverse effects could be seen. Any significant analgesic effect in addition to local anesthetics in a standard arthroscopic procedure is however doubtful. Maybe the lack of profound inflammation that seems to be a prerequisite for the analgesic action of morphine peripherally explains the absence of effects on inflammatory markers, metabolism and nociception. It cannot however be excluded that intra-articular administration of morphine is useful in a sub-group of patients with arthritic joint disease and manifest inflammation.

Cooling and compression effectively lowered skin, joint capsule and intra-articular knee temperature after knee arthroscopy. Inflammation was less profound and metabolism slowed down indicating less energy requirements of the cell and a tissue protective effect. Cooling and compression devices are simple to handle, reasonably cheap, well tolerated by the patients and probably without adverse effects. This supports a role for postoperative combined compression and cryotherapy after knee surgery.

To the best of my knowledge real-time local levels of glutamate were studied in synovial tissue for the first time in man. The results demonstrated high glutamate concentrations that diminished over time. This may confirm an important role for glutamate in local nociception and inflammation.

In our clinical setting intra-articular administration of morphine is not used routinely. According to our clinical routine, ketorolac is used in selected patients where no cartilage preserving surgery such as meniscus suturing, mosaicplasty or microfracturing of cartilage injuries is performed. Cooling and compression is used routinely after arthroscopic assisted ACL reconstruction.

In the future pre-, intra- and postoperative handling should be specifically tailored and possibly individualised in order to reduce the effects of the surgical trauma. To make this possible the detailed tissue reactions to surgery and results of available intervention strategies must be thoroughly studied. Microdialysis is an important tool for further studies of local metabolism and inflammation.
9 LIMITATIONS

The present studies have limitations in detecting differences in pain scoring because the number of patients was small and the study was primarily designed to evaluate the effects on inflammatory and metabolic compounds. Another limitation is that knee arthroscopy can be regarded as a minor surgical procedure, maybe without decisive pain response. All patients received bupivacain intra-articularly, which apart from affecting the pain response and diminishing differences between the groups in subjective pain variables, also probably affected the expression of inflammatory markers and metabolic compounds.

10 FUTURE STUDIES

The present thesis has answered some questions but also given rise to many new questions. This work will be continued with the aim to be able to better explain the role of glutamate in knee joint pain, the clinical significance of the proposed toxicity of local anesthetics and other pharmacological compounds working towards finding an optimal pre-, intra- and postoperative procedure in the future for enhanced recovery.
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