

DEPARTMENT OF EQUINE AND SMALL ANIMAL MEDICINE  
FACULTY OF VETERINARY MEDICINE  
UNIVERSITY OF HELSINKI

**Assessment of chronic pain  
and evaluation of three  
complementary therapies  
(gold implants, green lipped mussel and a  
homeopathic combination preparation)  
for canine osteoarthritis,  
using randomized, controlled,  
double-blind study designs**

Anna Hielm-Björkman

HELSINKI 2007



DEPARTMENT OF EQUINE AND SMALL ANIMAL MEDICINE  
FACULTY OF VETERINARY MEDICINE  
UNIVERSITY OF HELSINKI

**Assessment of chronic pain  
and evaluation of three  
complementary therapies  
(gold implants, green lipped mussel and  
a homeopathic combination preparation)  
for canine osteoarthritis,  
using randomized, controlled,  
double-blind study designs**

**ACADEMIC DISSERTATION**

*TO BE PRESENTED, WITH THE PERMISSION OF THE FACULTY OF VETERINARY  
MEDICINE OF THE UNIVERSITY OF HELSINKI, FOR PUBLIC CRITICISM IN  
AUDITORIUM XII, UNIONINKATU 34, 00100  
ON THE 15TH OF DECEMBER 2007, AT 10.00.*

HELSINKI 2007

**SUPERVISED BY:**

PROFESSOR RIITTA-MARI TULAMO  
DEPARTMENT OF EQUINE AND SMALL ANIMAL MEDICINE,  
SURGERY  
FACULTY OF VETERINARY MEDICINE  
UNIVERSITY OF HELSINKI, FINLAND

ASSOCIATE PROFESSOR MARJA RAEKALLIO  
DEPARTMENT OF EQUINE AND SMALL ANIMAL MEDICINE,  
PHARMACOLOGY  
FACULTY OF VETERINARY MEDICINE  
UNIVERSITY OF HELSINKI, FINLAND

**REVIEWED BY:**

PROFESSOR JOHN F INNES  
FACULTY OF VETERINARY SCIENCE  
DIVISION OF SMALL ANIMAL STUDIES, SMALL ANIMAL  
TEACHING HOSPITAL  
UNIVERSITY OF LIVERPOOL, UK

PROFESSOR STEVEN C BUDSBERG  
DEPARTMENT OF SMALL ANIMAL MEDICINE AND SURGERY  
COLLEGE OF VETERINARY MEDICINE  
UNIVERSITY OF GEORGIA, USA

**OPPONENT:**

PROFESSOR DUNCAN X LASCELLES  
DEPARTMENT OF SMALL ANIMAL SURGERY  
COLLEGE OF VETERINARY MEDICINE  
NC STATE UNIVERSITY, USA

ISBN 978-952-92-3198-0 (PAPERBACK)

ISBN 978-952-10-4444-1 (PDF)

HELSINKI UNIVERSITY PRINTING HOUSE 2007

## SUMMARY

The series of investigations presented in this thesis examined different methods of assessing chronic pain in dogs suffering from osteoarthritis (OA) and compared the effects of three different treatments. Data were obtained from two cohorts; 41 dogs with OA due to canine hip dysplasia (CHD) (I,III) and 61 dogs with OA due to CHD or elbow dysplasia (II,IV,V).

Questionnaires, veterinary evaluations, visual analog scales (VAS), plasma hormones, radiographs, and force plate evaluations were assessed as OA treatment outcome measures and/or measurements of chronic pain.

The results indicated that the multidimensional pain scale including 11 questions, each with five responses to choose from, was a valid and reliable tool for evaluating chronic pain. This Helsinki chronic pain index (HCPI) can be applied as an outcome measure in clinical trials where chronic pain is evaluated by owners.

Of the evaluated complementary therapies for chronic pain due to OA, all three indicated a positive treatment outcome. In the first trial, gold bead implants resulted in a significant positive treatment outcome for the treatment group. However, the placebo group in this study also improved significantly. A positive effect was seen in 53 to 63% of the placebo dogs and this unnormally high incidence of amelioration suggests that the placebo group may have got an effect of unintentional needle acupuncture. The results of this study are therefore controversial and treatment guidelines based on these findings cannot be given.

The second trial tested two ingestible OA remedies, green lipped mussel and a homeopathic low-dose combination preparation. Both treatments resulted in statistically significant positive treatment outcomes compared with placebo, but with the positive control (carprofen) being more effective than either of them. The results suggest that both tested treatments may be beneficial for chronic OA. To establish the true role of all these three treatments in outcome-based animal analgesia, more clinical trials, using larger cohorts, should be conducted. Possible of action mechanisms should also be studied.

*“Man who says it can't be done  
should not interrupt woman doing it”*  
-slightly modified Chinese proverb

This work is dedicated to the memory of my parents:

To my father,  
who taught me to keep an open mind  
but to question everything,  
and to  
my mother,  
who taught me  
how to handle 1000 things simultaneously,  
and love it.

# Contents

<b>SUMMARY</b> .....	5
<b>LIST OF ORIGINAL PUBLICATIONS</b> .....	11
<b>ABBREVIATIONS</b> .....	13
<b>1. INTRODUCTION</b> .....	15
<b>2. REVIEW OF THE LITERATURE</b> .....	19
2.1 Canine osteoarthritis (OA) .....	19
2.1.1 Cartilage structure .....	20
2.1.2 OA pathophysiology and biochemistry .....	20
2.1.3 Canine hip dysplasia (CHD) .....	22
2.1.4 Elbow dysplasia (ED) .....	23
2.2 Chronic pain assessment in dogs .....	24
2.2.1 Pain scales .....	25
2.2.1.1 Observational VAS scale .....	26
2.2.1.2 Multifocus/multifactorial descriptive scale (MDS scale) .....	27
2.2.2 Need for rescue analgesia .....	27
2.2.3 Veterinary evaluation .....	27
2.2.4 Hormones related to chronic pain .....	28
2.2.5 Radiographic changes .....	28
2.2.6 Force plate as a measure of weight bearing .....	29
2.3 Management of OA .....	30
2.3.1 Nonsteroidal anti-inflammatory drugs (NSAIDs) .....	30
2.3.2 Disease-modifying OA drugs (DMOAD) .....	32
2.3.2.1 Green lipped mussel (GLM) .....	33
2.3.3 Homeopathy .....	35
2.3.3.1 Homeopathic combination preparation (HCP) Zeel® ad us vet. ....	36
2.3.4 Acupuncture .....	38
2.3.4.1 Gold implantation in acupuncture points of the hip ..	41

<b>3. OBJECTIVES OF THE STUDIES</b> .....	43
<b>4. MATERIALS AND METHODS</b> .....	45
4.1 Dogs .....	45
4.2 Study protocols .....	46
4.3 Assessment of chronic pain and other clinical trial outcome measures .....	49
4.3.1 Owner evaluations: MDS questionnaire and VAS scales .....	49
4.3.2 Mobility evaluations by veterinarians .....	51
4.3.3 Plasma hormone assays .....	51
4.3.4 Radiographic examination .....	52
4.3.5 Force plate analysis .....	53
4.4 Statistical analyses .....	54
<b>5. RESULTS</b> .....	57
5.1 Pain/outcome assessing studies (I-II): .....	57
5.1.1 Owner evaluations (I,II) .....	57
5.1.1.1 MDS questionnaire and the Helsinki chronic pain index (HCPI) (I,II) .....	57
5.1.1.2 VAS scales (I) .....	59
5.1.2 Veterinary-assessed mobility index / locomotion (I) .....	60
5.1.3 Plasma hormone assays (I) .....	60
5.1.4 Radiographic examination (I) .....	60
5.2 Experimental treatment studies (III-V): .....	60
5.2.1 Gold implant study (III) .....	60
5.2.1.1 Owner evaluations (III) .....	60
5.2.1.2 Veterinary-assessed mobility index/locomotion (III) .....	61
5.2.1.3 Radiographic examination (III) .....	61
5.2.1.4 Complications and side-effects (III) .....	61
5.2.2 GLM and HCP studies (IV,V) .....	61
5.2.2.1 Owner evaluations (IV,V) .....	62
5.2.2.2 Veterinary-assessed mobility index (IV,V) .....	63
5.2.2.3 Force plate analysis (IV,V) .....	64
5.2.2.4 Intake of rescue NSAIDs (IV,V) .....	65
5.2.2.5 Comparing the tested treatments to carprofen (IV,V) .....	65
5.2.2.6 Complications and side-effects (IV,V) .....	66

<b>6. DISCUSSION</b> .....	67
6.1 Dogs .....	67
6.2 Variables chosen for outcome assessment .....	67
6.2.1 Owner evaluations .....	68
6.2.2 Mobility evaluation by veterinarians .....	71
6.2.3 Plasma hormone assays .....	71
6.2.4 Radiographic examination .....	72
6.2.5 Force plate analysis.....	72
6.3 Discussion on clinical research of OA .....	74
6.3.1 Common problems in clinical research of OA .....	74
6.3.2 Gold bead implants (III).....	76
6.3.3 GLM.....	78
6.3.4 HCP .....	79
6.3.5 Statistical methods .....	81
6.3.6 Working mechanisms of treatments used in this study .....	82
<b>7. CONCLUSIONS</b> .....	85
<b>8. EPILOGUE</b> .....	87
<b>ACKNOWLEDGMENTS</b> .....	89
<b>APPENDIX</b> .....	91
<b>REFERENCES</b> .....	95



## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals (I-V):

- I. Hielm-Björkman AK, Kuusela E, Liman A, Markkola A, Saarto E, Huttunen P, Leppäluoto J, Tulamo R-M, Raekallio M: Evaluation of methods for assessment of pain associated with chronic osteoarthritis in dogs *J Am Vet Med Assoc* 2003,222,1552-1558.
- II. Hielm-Björkman AK, Rita H, Tulamo R-M: The Helsinki chronic pain index (HCPI) – validation in dogs with osteoarthritis (*Submitted to J Am Vet Med Assoc* 27.7.2007).
- III. Hielm-Björkman A, Raekallio M, Kuusela E, Saarto E, Markkola A, Tulamo R-M: Double-blind evaluation of implants of gold wire at acupuncture points in the dog as a treatment for osteoarthritis induced by hip dysplasia. *Vet Rec* 2001,149,452-456.
- IV. Hielm-Björkman A, Tulamo R-M, Salonen H, Raekallio M: Evaluating complementary therapies for canine osteoarthritis. Part I: green-lipped mussel (*Perna canaliculus*). *Evid Based Complement Alternat Med* 2007 doi: 10.1093/ecam/nem136.
- V. Hielm-Björkman A, Tulamo R-M, Salonen H, Raekallio M: Evaluating complementary therapies for canine osteoarthritis. Part II: A homeopathic combination preparation (Zeel®). *Evid Based Complement Alternat Med* 2007 doi: 10.1093/ecam/nem143.

These original articles have been reprinted with kind permission from the American Veterinary Medical Association (I, II), the British Veterinary Association (III) and the eCAM at Oxford publications (IV, V).



## ABBREVIATIONS

AFOS = alkaline phosphatase  
ALAT = alanine aminotransferase  
AMG = Autometallography  
BMP = Bone morphogenetic proteins  
BUN = blood urea nitrogen  
BW = body weight  
CAM = complementary and alternative medicine  
CDMP = cartilage-derived morphogenetic protein  
CHD = canine hip dysplasia  
CI = confidence interval  
CIA = collagen induced arthritis  
CONSORT = consolidated standards of reporting trials  
COX = cyclooxygenase (e.g. COX-1)  
DHA = docosahexaenoic acid  
DJD = degenerative joint disease  
DMOAD = disease-modifying osteoarthritis drug  
DNIC = diffuse noxious inhibitory control  
EA = electroacupuncture  
EBM = evidence-based medicine  
ED = elbow dysplasia  
EPA = eicosapentaenoic acid  
ETA = eicosatetraenoic acid  
FDA = Food and Drug Administration  
FGF = fibroblast growth factors  
f-MRI = functional magnetic resonance imaging  
GABA = gamma-aminobutyric acid  
GAG = glycosaminoglycan  
GLM = green lipped mussel  
GRF = ground reaction force  
HCP = homeopathic combination preparation  
HCPI = Helsinki chronic pain index  
IGF-1 = insuline-like growth factor-1  
IFN- $\gamma$  = interferon  $\gamma$   
IL-1 = interleukin-1 (also IL-6, IL-17...)  
IVAS = International Veterinary Acupuncture Society  
LIF = leukemia inhibitory factor  
LOX = lipoxigenase (e.g. 5-LOX or simply 5-LO)  
MDS = multifactorial descriptive scale

MMP-1 = matrix metalloproteinase-1 (also MMP-3, -8...)  
NF $\kappa$ B = nuclear factor  $\kappa$ B  
NIH = National Institutes of Health (USA)  
NK = natural killer (cell)  
NMES = neuromuscular electric stimulation  
NO = nitric oxide  
NSAID = nonsteroidal anti-inflammatory drug  
OA = osteoarthritis  
PC = principal component  
PCA = principal component analysis  
PDGF = platelet-derived growth factor  
PDS = potential data-supported value  
PG = prostaglandin (e.g. PGE2)  
PSGAG = polysulphated glycosaminoglycan  
PUFA = polyunsaturated fatty acid  
PVF = peak vertical force  
RCT = randomized controlled trial  
ROM = range of motion  
SD = standard deviation  
SDS = simple descriptive scale  
TENS = transcutaneous electrical nerve stimulation  
TGF- $\beta$  = transforming growth factor- $\beta$   
TNF- $\alpha$  = tumour necrosis factor- $\alpha$   
VAS = visual analog scale  
W<sub>n</sub> = W for week and the subscript number for the week in question.  
W<sub>0</sub> is baseline.

# 1. INTRODUCTION

Canine osteoarthritis (OA) is frequently encountered in small animal practice; canine hip dysplasia (CHD) and elbow dysplasia (ED) are two common forms (Innes 2005). As both conditions are usually lifelong and degenerate rather than improve, it is of the utmost importance to treat these dogs. OA is also the most common of human musculoskeletal diseases and it is rapidly becoming a significant medical and financial burden to the world (Pelletier et al. 2006). Moreover, a second financial burden comes from treating people suffering from side-effects that come as a consequence of OA pain therapy. As a consequence, recommendations have been made to use more natural disease-modifying agents in the pain management of human OA rather than nonsteroidal anti-inflammatory drugs (NSAIDs) (Pendleton et al. 2000). To this end, more research is being conducted to find less detrimental medication and treatments to replace the long-term administration of NSAIDs or the renewable injections of corticosteroids, which today still are the widest used treatment options, although they are not ideal due to the risk of adverse reactions.

In evidence-based medicine (EBM), randomized controlled trials are crucial in the decision-making of which treatment to use, for doctors and veterinarians alike. Few of the new complementary OA treatments are registered drugs or treatments for animals, with many of them still lacking thorough testing and adequate clinical trials. There is, however, abundant research emerging in this field. In reviewing the Cochrane Library, which uses the EBM concept to do meta-analyses on recent randomized controlled trials (RCT) for different treatments of human OA, the following evaluations of new treatments for OA are presented: there is “convincing evidence for avocado-soybean unsaponifiables” (Little et al. 2003), “statistically significant improvement in all variables for electromagnetic field treatment for knee OA” (Hulme et al. 2003), “TENS (transcutaneous electrical nerve stimulation) and acupuncture-like-TENS are both shown to be effective in pain control over placebo” (Osiri et al. 2003), all 16 RCT trials showed that “glucosamine is both effective and safe” (Towheed et al. 2003), whereas “results are conflicting in different studies” when assessing low-level laser therapy for treating OA (Brosseau et al. 2003a). A review on homeopathy for osteoarthritis is expected to be published in the Cochrane library 2008, Issue 1 (Munar et al. 2007).

Complementary medicine (also referred to as alternative medicine or CAM) is a highly sensitive topic among many doctors and researchers, in

both human and veterinary medicine. Complementary research studies have often had poor study designs and therefore low credibility. Most of this type of research has been done as summed case studies by clinicians without adequate research competence and training. For some years now, increasingly more, and better quality, research in complementary and alternative medicine has been conducted in universities, large research centers, and even at the government level by both the European Union and the US Food and Drug administration (FDA). Also, poor research design is not exclusive to complementary medicine; in the same issue of the Cochrane Library on OA treatments, regarding a meta-analysis on NSAIDs for treating OA of the knee, the reviewers concluded: "In spite of the large number of publications in this area, there are few randomized controlled trials. Furthermore, most trials comparing two or more NSAIDs suffer from substantial design errors" (Watson et al. 2003).

As was seen from the Cochrane studies, many treatments are available for OA that could be evaluated also for dogs. Canine OA is a progressive and debilitating disease, very similar to the human disease, and the side-effects from NSAIDs in dogs are also notable (MacPhail et al. 1998, FDA 1999). Therefore, the need to evaluate new, less dangerous therapies for canine OA is the same as in the human disease. As canine OA is used as a model for human OA (Pond & Nuki 1973, Stoker et al. 2006) and as all of the treatments for the disease are available both for humans and dogs, the outcomes of these studies should also be of major interest for human medicine.

The alternative therapies most commonly used in veterinary medicine appear to be acupuncture, herbal medicine, and homeopathy (Hektoen 2005), together with nutraceuticals, a newer group that also may contain animal-like products. Although still subject to debate, acupuncture is gaining acceptance in academic medicine because its mechanisms of action can to some extent be scientifically explained (see reviews in Steiss 2001, Ma et al. 2005), and its clinical effects have been clinically evaluated and recommended for some conditions in human beings (ter Riet et al. 1990, NIH 1998). Herbal medicine and nutraceuticals are not theoretically incompatible with existing medical science, but documentation of the clinical effects for specific conditions has thus far been limited. This is, however, changing, as can be seen from the large number of recently published studies and reviews (DeHaan et al. 1994, Innes et al. 2000, 2003, Bauer 2001, Bierer & Bui 2002, Moreau et al. 2003, McCarthy et al. 2007). In contrast, the potential effects of the highly diluted homeopathic remedies cannot be explained in terms of current scientific theories, and thus these remedies are highly controversial in both human and animal medicine (Hektoen 2005). The three treatments tested here fall into each of these categories and will be reviewed together with the more conventional treatments for canine OA.

To be able to evaluate the clinical outcome of treatments for OA, measures evaluating pain or other OA symptoms are of the utmost importance. Pain is a subjective sensation and therefore should be assessed by the subject himself. For dogs, however, we must rely on veterinarians' and owners' views of the animal's abnormal locomotion or behavior. At the time of our first clinical trial, no validated canine chronic pain assessment scores were available.

This thesis evaluated relatively unknown OA treatments for dogs in rigorous, randomized, controlled, or double-controlled, double-blind trials. We also developed and evaluated pain assessment methods for dogs with chronic pain.



## 2. REVIEW OF THE LITERATURE

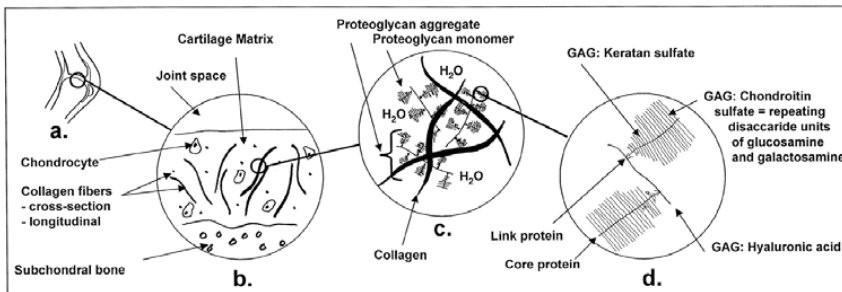
### 2.1 Canine osteoarthritis (OA)

Osteoarthritis (OA) is the most common arthropathy affecting dogs (Bennett & May 1995). An estimated 20% of the canine population in the United Kingdom and in USA suffer from OA (Moore et al. 2001). It is a disorder of movable joints characterized by degeneration of articular cartilage and the formation of new bone at joint surfaces or margins (Bennett & May 1995). The term OA indicates degenerative joint disease (DJD) with concurrent synovial inflammation, which, however, is not invariably present (Altman & Gray 1985, Bennett & May 1995). DJD is a term preferred by many clinicians, as it indicates a pathological process not always associated with inflammation. However, OA appears to be the term that is most commonly used in the veterinary literature (Vaughan-Scott & Taylor 1997) and will therefore be used throughout the thesis.

OA has been divided into two forms: primary and secondary OA. Primary OA is the result of defective articular cartilage structure and biosynthesis and is uncommon in dogs (Bennett & May 1995). Aspden et al. (2001) hypothesized that OA might in fact be a systemic disorder that affects the whole musculoskeletal system and involves altered lipid metabolism. Secondary OA results from abnormal forces acting on a normal joint (overweight, fracture, luxation, infection, crystal arthropathy, or immune mediated inflammation) or normal forces acting on an abnormal joint (abnormal joint conformation, osteochondrosis, hip and elbow dysplasia) (Bennett & May 1995, Innes 2005). Both in dogs and in humans, overweight has been shown to be a direct causative factor of OA, and losing weight significantly reduces the risk for OA (Felson et al. 1988, 1992, Kealy et al. 1992, 1997, 2000, Smith et al. 2006). Losing weight has significantly improved hind limb lameness in dogs with CHD (Impellizeri et al. 2000). However, it is still unclear whether the cause of OA is purely a mechanical overload or of metabolic origin, as OA changes exist also in nonload-bearing joints, such as human hands (Oliveira et al. 1995). Human and canine patients with early OA have a proliferation of poorly mineralized bone and an increased bone mineral density (Li & Aspden 1997, Chalmers et al. 2006). OA should be considered a disease process, the final common pathway for joint failure. Clinical manifestations of OA include pain and limited mobility in one or multiple joints.

### 2.1.1 Cartilage structure

Normal cartilage consists of a small number of chondrocytes embedded in matrix (**Fig. 1 b**). The matrix, comprising water, collagen, and proteoglycans, is formed by chondrocytes (**Fig 1 c**). The proteoglycan aggregates (**Fig. 1 d**) are made of numerous proteoglycan monomers bound to hyaluronic acid. Each proteoglycan is made up of several mucopolysaccharides called glycosaminoglycans (GAGs) (about 95%) attached to a core protein (about 5%), which in turn is joined to the hyaluronic acid molecule by a link protein. The GAGs in cartilage are chondroitin-4-sulphate, chondroitin-6-sulphate, hyaluronic acid, keratan sulphate, dermatan sulphate, and heparin sulphate. Chondroitin sulphate is composed of repeating disaccharide units of glucosamine and galactosamine. Glucosamine is either formed from a nutritional supplement or synthesized from glucose and amino acids. Galactosamine is formed from glucosamine by changing one of the hydroxyl groups (Heinegård & Sommarin 1987).



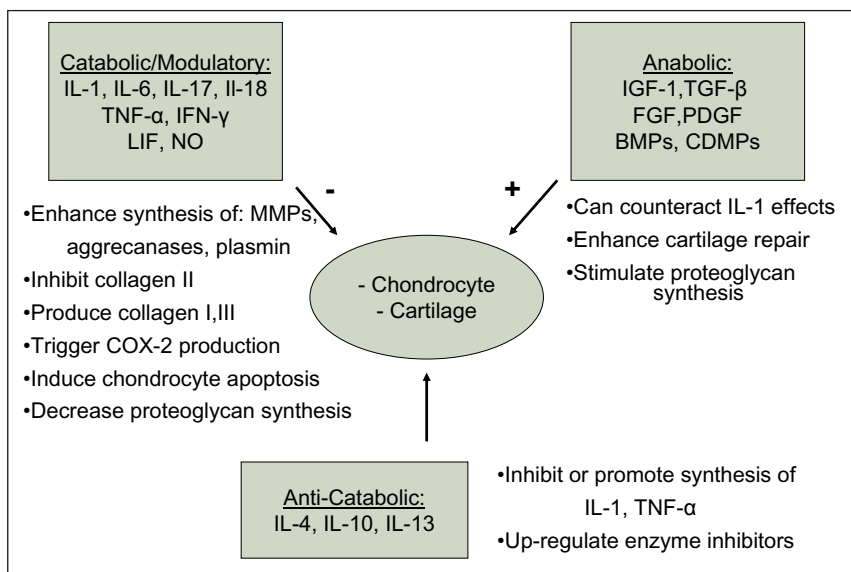
**Fig 1.** Components of normal cartilage

As the proteoglycan forms complexes with the hyaluronic acid, it acts as an osmotic trap to hold the water between the collagen strands (May 1994). Together, the water and the proteoglycan act as a shock absorber that enables cartilage to withstand normal loading forces (Clark 1991). As the cartilage is loaded, water is squeezed to the surface and the matrix is compressed. As the load is removed, water is reabsorbed by the cartilage and its shape is restored. Cartilage is not static but a living tissue and constantly regenerating; the chondrocytes are continually involved in normal anabolic repair processes of the matrix (Clark 1991).

### 2.1.2 OA pathophysiology and biochemistry

The exact mechanisms of cartilage degeneration are not fully understood. OA can be studied on at least three different levels: (1) gross joint and cartilage changes, (2) the destructive cellular enzymes released during inflammation (matrix metalloproteinases [MMP]) and, (3) the cellular and molecular triggers (cytokines and nitric oxide [NO]) (Millis 2005)(**Fig. 2**). OA results from catabolic processes exceeding anabolic processes (Clark

1991). Articular cartilage undergoes softening and fibrillation early in OA. Cartilage fragments and matrix degradation products are released from the damaged cartilage. When degeneration exceeds regeneration, it will lead to synthesis of proteoglycans with an abnormal biochemical structure, loss of proteoglycans, and an abnormal cartilage structure (Clark 1991). Certain types of lymph node cells increase in collagen-induced arthritis (CIA), indicating that T cells and B cells are key participants in OA pathogenesis (Yim et al. 2007). Activated T cells promote disease progression by inducing secretion of proinflammatory cytokines from macrophages and synovial cells. In early OA, several of these destructive cytokines (IL-1, IL-6, IL-17, IL-18, TNF- $\alpha$ , LIF, and IFN- $\gamma$ ) trigger the release of enzymes that influence proteoglycan synthesis and are involved in cartilage degradation (May 1994, Miossec 2004, Yim et al. 2007). Cartilage degeneration leads to localized areas of soft cartilage, flaking, fissures, and decreased load-bearing capacity (Bennett & May 1995). Other anabolic cytokines (growth factors IGF-1, TGF- $\beta$ , FGF, PDGF, and CDMP) try to counteract cartilage degeneration (Goldring & Goldring 2004). The synovial fluid quality decreases in inflammation due to defective hyaluronic acid synthesis and increased catabolism. This leads to decreased lubrication and additional cartilage trauma. Hypoxia results in lactate accumulation in the synovia and a low pH. Proteoglycans and type II collagen can further act as antigens when released into the synovia. They provoke an inflammatory response, releasing proteinases, prostaglandins (PGs), cytokines (IL-1, TNF- $\alpha$ ), and free radicals, such as NO, all of which directly or indirectly catabolize cartilage, bone, and hyaluronic acid (Goldring & Goldring 2004). IL-1 triggers production of cyclooxygenase-2 (COX-2) in chondrocytes, inhibits synthesis of type II collagen, which is essential to articular cartilage, and stimulates type I and III collagen, contributing to fibrosis (Goldring & Goldring 2004). NO has a negative effect on chondrocytes as it activates catabolic enzymes, the metalloproteinases (MMP-1= collagenase, MMP-3= stromelysin, and MMP-8= gelatinase), decreases collagen and proteoglycan synthesis, and induces chondrocyte apoptosis (Goldring & Goldring 2004). 1 and 2 aggrecanases (ADAM-TS 4 and -5) are similar to MMPs and the main enzymes responsible for the aggrecan catabolism in canine OA (Glasson et al 2005, Innes et al. 2005). In response to cellular necrosis and trauma, the subchondral bone and the zone of calcified cartilage, remodels and thickens (Daubs et al. 2006), altering their mechanical compliance. This again increases load-bearing stresses on the articular cartilage that decrease in thickness and lead to cartilage failure (Daubs et al. 2006).



**Fig.2.** Simplified schematic representation of the cytokine regulated pathogenesis of OA (a modified version of a slide from Millis 2005, with his permission, compiled from research findings presented in the text (mainly from Sandell & Aigner 2001, Goldring & Goldring 2004). For abbreviations see list at p.13-14.

### 2.1.3 Canine hip dysplasia (CHD)

Canine hip dysplasia (CHD) is a disease in dogs that causes laxity, abnormal development and, arthritis of the hip joint (Bennett & May 1995). The clinical consequences of CHD are extremely variable from dog to dog.

CHD is an abnormal development or growth of the coxofemoral joint, usually bilateral (Brinker et al. 1990). It is manifested by varying degrees of laxity of surrounding soft tissues, instability, and malformation of the femoral head and acetabulum, eventually leading to OA (Smith & McKelvie 1995). The hips are normal at birth, but failure of muscles and skeleton to mature together at the right time results in joint instability (Bennett & May 1995). The prevailing hypotheses are that the incidence of CHD can be reduced by restricting food intake (Smith et al. 2006) and the growth rate of puppies. Excessive calcium, total energy, and/or protein consumption at an early age has an influence on the disease, with overweight (Smith et al. 2006) and too heavy exercise (Black 1988, Cardinet et al. 1997), also playing a role. CHD has a polygenic mode of inheritance, and thus genetic selection will help to improve hip quality (Leighton 1997). In Finland, some breeds continue to have a high proportion of this disease: German Shepherd dogs 44%, Golden Retrievers 39%, Berner Sennen dogs 52% (Official Statistics of the Finnish Kennel Club 1988-2007), even after 44 years of systematic radiographic selection of only mildly affected or CHD-free individuals for breeding. Also, as radiographs of individuals with severe OA changes often

are not submitted for official evaluation, the true incidence is probably much higher (Paster et al. 2005). A study from USA has shown that radiographs with normal appearing hips were 8.2 times more likely to be sent to the Orthopaedic Foundation for Animals (OFA) than radiographs of non-normal hips, and that 78% of Golden Retriever hip radiographs that were not submitted were abnormal (Paster et al. 2005).

The earliest clinical sign of OA due to CHD is pain at full hind limb extension. Clinical signs of more advanced arthritis include loss of extension and abduction, muscle atrophy, and sometimes, an indistinct Ortolani sign (Montgomery 1998). The clinical presentation of CHD has been divided into two forms (Smith & McKelvie 1995): a severe form and a chronic form. The severe form typically appears between 5 and 12 months of age and shows signs of marked debilitating lameness, such as a noticeably abnormal gait, pain, low exercise tolerance, reluctance to go up and down stairs, atrophy of thigh muscles, occasionally an audible click when walking, and sometimes, if very severe, an obviously increased intertrochanteric (rump) width (Smith & McKelvie 1995). The chronic form, however, comprises the vast majority of cases. Dogs affected with this form can be totally asymptomatic, only mildly painful, or severely painful and disabled, particularly after periods of rest following excessive exercise or unaccustomed activity. This form often becomes evident with age and is characterized by a slow worsening of such signs as waddling gait, “bunny-hopping” when running, stiffness, slowness, reluctance to walk stairs, prefers to sit, slowness when rising, and excessive circling before lying down (Brinker et al. 1990). The Ortolani sign is rarely present owing to the shallowness of the acetabulum and fibrosis of the joint capsule (Brinker et al. 1990). Although less acute and debilitating than the severe form, the chronic form can progress to marked disuse and severe muscular wasting (Smith & McKelvie 1995). The clinical signs in chronic CHD are due to progression of OA (Smith & McKelvie 1995). Cold, damp, obesity and prolonged exercise often worsen signs of lameness (Bennett & May 1995).

A tentative diagnosis can be made on the basis of history, clinical signs, and palpation. A definitive diagnosis is, however, made only when the hip joint shows characteristic radiographic signs of CHD. The radiographs are taken with the dog sedated, either in a supine position with the limbs fully extended and the stifles mildly internally rotated or with the limbs in a froglike position (e.g. using the PennHip method). In Finland, the limbs are fully extended and a 5 point evaluation is used (FCI 1991); A= no signs of CHD, B= close to normal hip joints, C= mild CHD, D= moderate CHD, and E= severe CHD.

#### **2.1.4 Elbow dysplasia (ED)**

Elbow disease and elbow dysplasia (ED) are both umbrella terms for at least four different elbow pathologies; fragmentation of the medial coronoid

process, osteochondrosis dissecans of the medial humeral condyle, ununited anconeal process, and elbow incongruity. No scientific evidence indicates that these are true dysplasias, and it is probably unjustified to group such different entities with different etio-pathogeneses under the same name (Innes 2005). However, the term has been adopted internationally and will be used here as well.

As with CHD, ED either includes OA changes or not. At the stage when radiographic changes are seen, OA is mostly already present (Bennett & May 1995). In Finland, the percentages of this disease by radiographic screening are high e.g. in: Golden Retrievers 27%, Berner Sennen dogs 43%, Rottweilers 51% (Official Statistics of the Finnish Kennel Club 1988-2007).

The etiology of ED is on the whole unknown, but depending on which type of primary disease is referred to, genetic disposition, abnormal ossification, trauma, overloading due to overweight or exercise, possible malformation of the joint bone surfaces, nutritional excess, and metabolic reasons are all possible (Bennett & May 1995).

## 2.2 Chronic pain assessment in dogs

Since the first guidelines for recognition of animal pain (Morton & Griffiths 1985, Sanford et al. 1986), the acceptance for animals experiencing subjective pain in a similar way as humans, has grown in recent years (ACVA 1998, Lascelles & Main 2002, Robertson 2002, Rutherford 2002). Canine pain behavior can be divided into three categories. The first category consists of genetically predisposed pain responses common for all dogs and most other mammals, such as avoiding the triggering pain, physiological responses (pupils, heart rate, breathing rate), and screaming with acute pain (Morton & Griffiths 1985, Sanford et al. 1986, ACVA 1998). There are, however, marked differences both between and within species in pain behavior (Sanford 1992, Dobromylskyj et al. 2000). In the canine species, human genetic manipulation through breeding has resulted in very different dog breeds with very different behaviors, characters and appearances, e.g. guard dogs, competing runners, shepherds, heelers, hunting dogs, and companion dogs. As a consequence, there are more typical pain responses related to different dog breeds than among the same species of other animals; certain dog breeds are more stoic, while others are “whimpers” (Dobromylskyj et al. 2000). Moreover, distinct individual differences exist (Dobromylskyj et al. 2000).

The second category contains socially acquired pain responses. As humans seem to learn their pain behavior from their parents at a very early age (Sargent & Liebman 1985), dogs may also learn their pain behavior from their owners, as they would have learned from their own species,

had they lived in a dog pack (Dobromylskyj et al. 2000). Some owners encourage their dogs to show pain, others do not.

The third category of pain behavior is the ability to shift the two former pain responses. Dogs likely can set their behavioral patterns with such rigidity that these can override the genetic autonomic responses and muscle reactions normally triggered by pain (Wall 1992). These patterns can be used to show more pain – or less. All dogs can become “very lame” if they learn that they will gain more attention and perhaps titbits from certain members of the family. On the other hand, a dog showing severe pain symptoms in a normal setting may show no signs of pain when, for instance, taken out for a hunt or to a clinic (Dobromylskyj et al. 2000, Flecknell 2000).

Wall (1992) also points out the necessity of understanding a particular animal’s relationship with its environment at a particular time. The absence of the owner, the awkward smells, and the sounds of other animals may influence how or if an animal shows signs of pain (Dobromylskyj et al. 2000). Because of this shifting of the dog’s pain responses, it has been suggested that the owner’s observations should be considered in pain assessment (ACVA 1998, Hardie 2000, Wiseman et al. 2001) and used to evaluate treatment outcome in clinical research. The owner has a closer relationship than the researcher with the animal and should therefore be able to detect subtle changes in the dog’s mood and behavior in the normal environment. When owners evaluate pain in their dogs, they work as “proxies” and they observe someone else’s pain using observational scales. As several researchers recently have pointed out, outcome based veterinary medicine still lacks reliable validated outcome measures (Schulz et al 2006, Cook 2007, Kapatkin 2007a). When this problem will be tended to, it will enable us to draw more accurate conclusions when evaluating different treatments, for example for painful musculoskeletal diseases such as osteoarthritis (OA). Therefore, it is of utmost importance to now validate and test reliability for old and new chronic pain outcome measures.

### **2.2.1 Pain scales**

The scales used to assess pain in dogs are similar to those used in people: (1) visual analog scale (VAS); a response is indicated along a 10-cm continuum (Revill et al. 1976, Conzemius et al. 1997, Holton et al. 1998), (2) numerical/numeric rating scale (NRS), which has the numbers 1-10 written successively from left to right and where the assessor circles the number that seems to correspond best to his evaluation (Conzemius et al. 1997, Holton et al. 1998), (3) simple descriptive scales (SDS) which offer several (usually 3-5) written answers, often corresponding to degree of severity (Holton et al. 1998) - in some articles (e.g. Conzemius et al. 1997, Quinn et al. 2007) the term NRS has been used for questions that more typically would have been called SDS questions, which has lead to some confusion -

and (4) variable rating scale (VRS) (Hardie 2000), multifactorial pain scale (MFPS) (Firth & Haldane 1999, Dobromylskyj et al. 2000) and multifocus/multifactorial descriptive scale (MDS) that are close to synonyms and all contain a number of SDS questions relating to different aspects of pain (Hardie 2000). Attempts have been made to compare various methods of scoring or assessing pain in dogs, especially acute pain that develops after surgery (Conzemius et al. 1997, Holton et al. 1998, Firth & Haldane 1999, Holton et al. 2001). The science that validates scales is called psychometric statistics (Streiner 1993). Before researchers use a test, they will want to know that it is both valid and reliable (Carmines & Zeller 1979) but thus far chronic pain outcome measures have been very sparsely evaluated. There are many ways to test this and no one single test can unequivocally “prove” its worth, but together, tests strengthen a scale or an index (Carmines & Zeller 1979). The methods chosen are partly due to how data is gathered and partly due to the researchers’ preference. A short description of the terms used is given in **Appendix 1**. Pain scales were first used only by medical personnel such as veterinarians and research nurses (Conzemius et al. 1997, Welsh et al. 1997, Holton et al. 1998, 2001, Firth & Haldane 1999, Morton et al. 2005).

### **2.2.1.1 Observational VAS scale**

The observational pain VAS has a single 10-cm continuum. The left endpoint signifies “no pain”, whereas the right endpoint signifies the “worst possible pain”. The observer places a mark on the line corresponding to his/her view of the patient’s pain intensity. The VAS pain score is the distance to the nearest millimeter, between the mark and the left end of the scale (Varni et al. 1987). In a human study, the VAS for constant or chronic pain was deemed reproducible, a good correlation existed between repeated ratings of a recalled pain distant in time, and changes in ratings were likely to be real changes of opinion (Revill et al. 1976). Because of its strengths as a self-report measure, its ease of use, its good reliability and validity, its low cost, and its being a metric measure that enables parametric testing, the pain VAS was introduced for observational chronic pain assessment in human medicine (Varni et al. 1987, Huijjer Abu Saad & Uiterwijk 1995). In a review (van Dijk et al. 2002) evaluating an observational pain VAS for pediatric pain assessment, with professionals and parents evaluating small children (resembling owners evaluating their dogs’ pain), the validity was evaluated and the correlation coefficient of the children’s self-rated VAS compared with the professional proxies VAS ranged from 0.23 to 0.85 (median 0.53) and compared with parents’ VAS it ranged from 0.46 to 0.83 (median 0.70), in relatively small samples (n=13-46). The correlation coefficients between the observational VAS and other pain instruments ranged from 0.42 to 0.86 (median 0.68). Both parents and physicians tended to over-report chronic pain (Varni et al. 1987, Huijjer Abu Saad & Uiterwijk 1995).

Veterinarians and research assistants familiar with canine pain have used this human tool for acute and postoperative canine pain assessment in dogs (Conzemius et al. 1997, Holton et al. 1998) and chronic lameness in sheep (Welsh et al. 1993), but at the outset of our research we knew of no studies on an owner used pain VAS. Innes and Barr (1998) introduced an owner reported VAS tool for outcome assessment after knee surgery, in dogs.

### **2.2.1.2 Multifocus/multifactorial descriptive scale (MDS scale)**

The MDS contains a number of SDS questions relating to different aspects of pain. Here different variables can have either the same or different weights (Hardie 2000). Wiseman et al. (2001) were the first to report a preliminary study involving unstructured interviews with 13 owners of dogs with chronic pain. All owners reported some changes in their dogs' behavior and most reported some change in demeanor. Six veterinarians were also questioned about how they assess chronic pain, and they reported similar changes in canine behavior. Only recently have researchers validated owner used MDS scales. (Wiseman-Orr et al. 2004, 2006, Brown et al. 2007b).

In a comparative MDS questionnaire, questions are posed to compare a variable with something, usually the baseline or the time before treatment. These typically include 3-5 answers on a scale of having changed for the better, stayed the same, or changed for the worse (Gibson et al. 1980, Bollinger et al. 2002, Väisänen et al. 2004, Pollard et al. 2006, Jaeger et al. 2007). At the end of the trial period or at follow-up, questions regarding owner satisfaction or trial outcome has been used (Jaeger et al. 2007). Scales where the owners have to guess what treatment their animal received and questions about if owners would gladly continue their animals treatment, have also been used (Jaeger et al. 2007).

### **2.2.2 Need for rescue analgesia**

As it is ethically necessary to alleviate severe pain, the amount of additional medication needed, often referred to as rescue analgesics, can be used as a measure of treatment success (Sanford et al. 1986, Innes et al. 2003, Hamunen & Kalso 2005). Drop-out rate has also been used as a measure of effectiveness in human studies (Caughey et al. 1983).

### **2.2.3 Veterinary evaluation**

Evaluating chronic pain due to OA in dogs has been done using very different means of measuring: Lameness and weight bearing are commonly used (Holtzinger et al. 1992, Vasseur et al. 1995, Borer et al. 2003, Peterson & Keefe 2004). Abnormalities of the locomotor system can be scored, for example, limb circumference as a measure of atrophy (Dobromylskyj et al. 2000, Millis 2004), decreased limb range of motion (ROM) of extending or flexing joints (Holtzinger et al. 1992, Vasseur et al. 1995, Millis 2004),

swelling (Borer et al. 2003), crepitus (Holtsinger et al. 1992), pain from palpation (Holtsinger et al. 1992, Vasseur et al. 1995, Borer et al. 2003), or willingness to hold up a contralateral limb (Vasseur et al. 1995). No research showing validity or reliability of these methods is to our knowledge, available.

#### **2.2.4 Hormones related to chronic pain**

Concentrations of various hormones have been used to assess stress and pain in animals (ACVA 1998). Plasma adrenaline, noradrenaline,  $\beta$ -endorphin, cortisol, and vasopressin concentrations are known to increase in stressful situations such as trauma and surgery (Desborough 2000). However, no information regarding the change in concentration of any of these hormones in response to chronic pain in dogs is available. In horses that were expected to have severe postoperative acute pain,  $\beta$ -endorphin concentration was shown to increase (Raekallio et al. 1997). In a study by McCarthy et al. (1993), however, one control horse that suffered from painful chronic OA had decreased  $\beta$ -endorphin concentration. Almay et al. (1978) observed that organic pain in humans resulted in decreased cerebral spinal fluid endorphin concentrations. In Ley et al. (1992), sheep with chronic foot rot-associated lameness had increased plasma adrenaline and noradrenaline concentrations, compared with those of control sheep. In another study, the investigators showed no consistent changes in vasopressin concentration in chronically lame sheep, but cortisol concentration was decreased compared with controls (Ley et al. 1991). In a later study with a greater number of sheep, an increase in plasma cortisol concentration was noted in lame sheep, but no correlation was present between disease severity and cortisol concentration (Ley et al. 1994).

#### **2.2.5 Radiographic changes**

It is generally accepted that the clinical status or the amount of pain of an animal cannot be predicted from the pathologic changes seen on radiographs (Dobromylyskij et al. 2000). Kealy et al. (2000) have shown that the most common finding in dogs with OA of the hip was periarticular osteophytes in the proximal aspect of the femur. At the time of our trial, we found no studies where radiographic changes within the coxofemoral or elbow joint would have been correlated with pain assessment scales. Recently some studies have been published on radiographic OA and limb function in the stifle (Gordon et al. 2003) and in the shoulder (Åkerblom & Sjöström 2007). Radiological abnormalities and changes in locomotion that result from chronic pain associated with disease of the hip joint in dogs are, however, well documented (Smith 1997, Slocum & Slocum 1998). The radiographic features of advanced OA are well documented. This is the most commonly used method for diagnosing OA, showing joint

space narrowing, subchondral bone sclerosis, subchondral cyst formation, marginal osteophytes (Creamer & Hochberg 1997), joint deformity with preservation of articular margins, proliferative and lytic changes at the attachment sites of the joint capsule and the supporting ligaments, and partial to complete ankylosis (Gielen 2005). It is an excellent imaging technique for bony structures, but is poor for soft tissue structures. Another drawback is that as a two-dimensional technique it superimposes structures and can therefore mask marked changes (Gielen 2005). Plain radiographs can confirm a diagnosis, but the absence of changes does not exclude the presence of OA. Early OA is difficult to diagnose, as no visible radiological changes are yet apparent (Bennett & May 1995).

### **2.2.6 Force plate as a measure of weight bearing**

The most objective pain assessment method for dogs with chronic limb pain now available, is measurement of ground reaction forces (GRF) by force plate analyses, where it is postulated that a dog will put less weight on a limb if it is painful (Anderson & Mann 1994). Force plate analysis is viewed as the gold standard for evaluation of lameness (Quinn et al. 2007). The used force plates measure three orthogonal forces: mediolateral ( $F_x$ ), craniocaudal ( $F_y$ , also referred to as the braking and propulsive force), and vertical ( $F_z$ , peak or mean vertical force [PVF] and vertical impulse). Dogs normally carry 60% of their bodyweight on the forelimbs and 40% on the hind limbs (Budsberg 1987).

The force plate has been used to evaluate GRF in dogs with CHD; these dogs have significantly reduced vertical forces in the hind limbs and stride length is increased, but velocity, maximal foot velocity, stance duration, and stride frequency do not differ between CHD and clinically normal dogs (Bennett et al. 1996). Force plate has been used to evaluate treatments of OA of the hip (Vasseur et al. 1995, Budsberg et al. 1996, 1999, 2001, Moreau et al. 2003) and elbow joints (Bouck et al. 1995, Vasseur et al. 1995, Theyse et al. 2000, Moreau et al. 2003). The best variables for these conditions were considered to be PVF and vertical impulse. Vertical impulse was found to be a better indicator of improvement than the PVF and has often been selected as the primary response variable (Budsberg et al. 1996, 1999, 2001). Trotting velocities of 1.6-1.9 m/s (Budsberg et al. 1996, 1999, Kapatkin et al. 2007b), 0.8-2.1 m/s (Bennett et al. 1996), 1.45-2.05 m/s (Trumble et al. 2004, 2005), 1.8-2.3 m/s (Allen et al. 1994), 1.5-2.25 m/s (Jevens et al. 1996) and acceleration variation of  $\pm 0.5 \text{ m/s}^2$  (Budsberg 1996, 1999, Kapatkin et al. 2007b) have been used. The mean of 3-6 valid runs is typically used, as variation between runs can be very large (Jevens et al. 1996, Tano et al. 1998, Budsberg et al. 1999). To hide the force plate from the dogs, it is usually mounted into the floor or into a wooden walkway of 10-15 m that is covered with a rubber mat (Anderson & Mann 1994). Photocells that measure velocity and acceleration are mounted

beside the walkway (Budsberg et al. 1999). When force plate analysis has been compared to other measures of treatment outcome the results have not always been equal; in a trial by Vasseur et al. (1995) owners evaluated 38% of the placebo dogs to get better, veterinarians 26% of the dogs and the force plate showed 56% of the dogs to have become better.

## 2.3 Management of OA

So far, one should not talk about treating OA, but managing it, as no cure is known. A very early stage of OA has recently been identified where the disease process potentially still is reversible (Stoker et al. 2006). As a result, some researchers think that new therapies targeting the pathophysiology of OA will, with time, give us a definitive cure for OA (Pelletier et al. 2006). In the meantime, most doctors will use one of the two traditional ways to manage OA: pharmacological, using mainly NSAIDs or corticosteroids, or surgical (Kapatkin et al. 2002). However, since OA now is seen as a more complex ongoing process, a multimodal approach has been suggested and other approaches for different stages of the disease has also been introduced (Lascelles & Main 2002, Millis & Levine 2002, Pascoe 2002, Carmichael 2005). All dogs with OA should also avoid overweight and have regular exercise (Brosseau et al. 2003b, Fransen et al. 2003, Carmichael 2005).

### 2.3.1 Nonsteroidal anti-inflammatory drugs (NSAIDs)

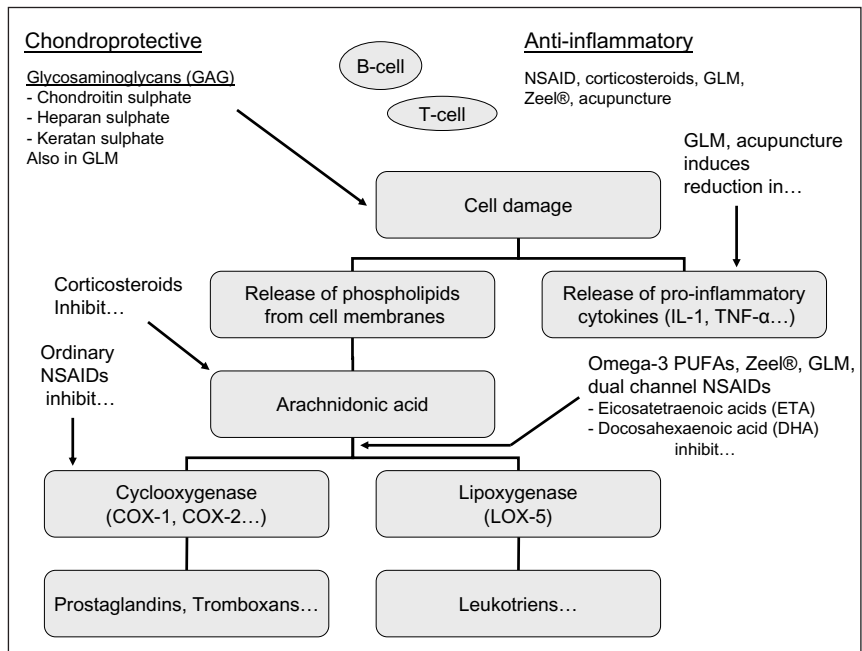
Nonsteroidal anti-inflammatory drugs (NSAIDs) were introduced for veterinary use in the form of sodium salicylate at the end of the 19<sup>th</sup> century (Lees 2005). For canine OA, carprofen and meloxicam are the two NSAIDs mostly used at present. As carprofen was used in our studies, it will be presented here.

Most NSAIDs inhibit the cyclooxygenase pathways COX-1 and COX-2 (**Fig. 3**), one or both, but their exact mechanism of action is still not fully understood (Fox & Johnston 1997, Lees 2005). COX-1 inhibition produces toxic effects, as COX-1 is a constitutive enzyme present in most cells of the body. COX-1 is responsible for inhibition of synthesis of pro-inflammatory mediators, such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), and also for many physiological functions in the body, including gastro- and reno-protection and blood clotting. Current evidence suggests that up to 95% PGE<sub>2</sub> inhibition may be required for effective suppression of lameness in OA dogs (Lees 2005). This COX-1 inhibition leads to potentially severe side-effects, such as renal toxicosis and irritation of the gastrointestinal tract, and possibly also to severe hemorrhagic ulcers and death (MacPhail et al. 1998, FDA 1999). In the 1998 annual report from the FDA in the United States, 43.4% of reports of adverse effects of drugs from all animals, indicated Rimadyl® (carprofen) for dogs as the suspected drug (FDA US ADE Report 1999).

Carprofen has also been found to trigger hepatic toxicosis, especially in Labradors (MacPhail et al. 1998). Other studies, however, have found no side-effects for carprofen (Holtsinger et al 1992, Raekallio et al. 2006).

COX-2 inhibition produces therapeutic effects, as COX-2 is an inducible enzyme present at sites of inflammation and is responsible for producing pro-inflammatory mediators. COX-2 is also recognized as a constitutive enzyme in brain, kidney, ovary, uterus, ciliary body, and bone (Lees 2005). Complete inhibition of COX-2 over long periods might therefore lead to abortion, fetal abnormalities, delayed healing of bone and soft tissue, cardiovascular problems, and renal toxicity. The cardiovascular events in humans due to COX-2-inhibiting NSAIDs support this speculation (Lees 2005). Further, COX-1 has been suggested to contribute to the synthesis of pro-inflammatory PGs. Both COX-1 and COX-2 inhibition might therefore be required for optimal efficacy (Lees 2005).

A newer type of NSAID is the dual inhibitor, inhibiting both COXs and 5-lipoxygenase (also referred to as 5-LO, 5-LOX, or LOX), thus blocking the synthesis of both PGs and leukotrienes. Its advantage is greater gastrointestinal, hepatic, and renal tolerance (Lees 2005).



**Fig. 3.** The points of action for some chondroprotective and anti-inflammatory products (compiled from research findings presented in the text).

Variation in pharmacokinetics and pharmacodynamics between dog breeds and individual animals occurs between drugs but also for the same drug, explaining the individual differences commonly encountered by clinicians and owners in therapeutic response and tolerance (Lees 2005).

Carprofen triggers a clinical response very quickly, that vanishes rapidly upon discontinuing the drug, as the half-life is merely 8 hours (Fox & Johnston 1997).

### **2.3.2 Disease-modifying OA drugs (DMOAD)**

OA is still a non-curable disease that gradually deteriorates to an end-stage disease. It is important to intervene as early as possible to reduce escalation of the pathology as the more advanced the condition is, the more difficult it is to treat (Carmichael 2005). As the pathophysiological events associated with OA are becoming increasingly understood, new therapies that target a specific pathway have emerged (Pelletier et al. 2006). Articular cartilage, subchondral bone, synovial fluid, and synovium of affected joints can be modified with “slow-acting drugs of OA”, “disease-modifying agents” or “disease-modifying osteoarthritis drugs” (DMOAD) (Carmichael 2005, Aragon et al. 2007).

The most attractive new therapeutic targets for the development of DMOAD are (1) cytokines (especially IL-1 $\beta$ ) (**Fig. 2**), NO, reactive oxygen species and eicosanoids (**Fig. 3**) to target the inflammatory process, (2) MMP-13 and Aggrecanase-2 to target cartilage degradation and (3) bisphosphonates to target subchondral bone remodelling (Pelletier et al. 2006). Glasson et al. (2005) showed that deletion of active ADAM-TS-5 prevents cartilage degradation in a murine model of osteoarthritis. In gene therapy OA can be treated by controlling the expression of a number of genes that are responsible for the synthesis of factors involved in cartilage degradation and/or those that promote cartilage repair (Gelse et al. 2005). Chan et al. (2006) showed that glucosamine and chondroitin sulfate *in vitro* inhibit the expression of MMPs and ADAM-TSs and increase the expression of one of their natural inhibitors, TIMP-3.

Evidence has implicated IL-1 $\beta$  as being the principal cytokine responsible for the signs and symptoms of inflammation in OA (Goldring & Goldring 2004). Compounds like rhenin (from diacerein) that inhibit IL-1 synthesis and activity have shown improvement of OA symptoms as it reduces articular cartilage damage (Pelletier et al. 2000). MMP inhibitors such as doxycycline are currently tested for OA indications (Brandt et al. 2005). Medications that have a bone anti-resorptive effect (oestrogen, raloxifene and alendronate) have been tested for OA but the results are still inconclusive (Pelletier et al. 2006).

Another target is boosting components of cartilage matrix. In the form of injections given parenterally, polysulphated glycosaminoglycans (PSGAG), hyaluronic acid and Ca- or Na-pentosan polysulphate has been studied on dogs (DeHaan et al. 1994, Aragon et al. 2007, Budberg et al. 2007). Peroral alternatives include nutraceuticals that mainly work through glycosaminoglycans (GAG) or their components, vitamins, minerals, and polyunsaturated fatty acids (PUFA) (Bauer 2001, Aragon et al. 2007). Of

the five GAGs present in cartilage tissue, chondroitin sulphate (primarily extracted from shark, bovine, or poultry tissues and green lipped mussel) or its constituent glucosamine (primarily extracted from chitin; the exoskeleton of crabs, shrimps, lobsters in the form of glucosamine sulphate or hydrochloride) are the most commonly used, together with omega-3 fatty acids (n-3 PUFAs) (Curtis et al. 2004). Glucosamine hydrochloride is readily absorbed in the intestine (up to 98%) (Senikar et al. 1986). Glucosamine sulphate has been shown to reduce symptoms of OA in humans in both single-blinded (Crolle & DiEste 1980, D'Ambrosio et al. 1981) and in experimental or double-blinded trials (Drovanti et al. 1980, Pujalte et al. 1980, Reichelt et al. 1994, Qiu et al. 1998, Clegg et al. 2006) and in dogs (Johnson et al. 2001, McCarthy et al. 2007). One trial has reported no effect in humans (Rindone 2000) and one in dogs (Moreau et al. 2003). In a Cochrane review of human RCTs where glucosamine was compared to NSAIDs, glucosamine was found to be superior in two and equivalent in two (Towheed et al. 2003). Chondroitin sulphate has been shown to reduce pain, increase joint mobility, and induce healing of the joints of people with OA (Pipitone et al. 1992, Morreale et al. 1996, Uebelhart et al. 1998, Verbruggen et al. 1998, Clegg et al. 2006). The role of PUFAs will be reviewed under GLM.

### 2.3.2.1 Green lipped mussel (GLM)

The green lipped mussel (GLM) is a DMOAD with multiple targets. However, its mechanism of action is not entirely understood (Servet et al. 2006). GLM products are a rich source of nutrients, including GAGs, such as chondroitin sulphates, vitamins, minerals, and omega-3 series PUFAs (Halpern 2006) (**Table 1**).

**Table 1.** Content of a 100% GLM product, typical analysis. 1 capsule often contains 500 mg. (Technyflex/Lyproflex test certificate 2002)

~50% protein	
~10% fat	
	Total Omega-3s: 18 mg/1 g, here the 3 main ones:
	1 mg eicosatetraenoic acids (ETA)/1 g
	8,8 mg eicosapentaenoic acid (EPA)/1 g
	5,5 mg docosahexaenoic acid (DHA)/1 g
	} the n-3 PUFAs
~17 % carbohydrates	
	Proteoglycans 30%. GAGs: 11-15%, predominantly chondroitin sulfate
~5% moisture	
~18 % ash	
	Minerals: B, Ca, Cu, Cr, I, Fe, Mn, Mg, K, P, Na, S, Se, Ta, Zn
	Vitamins: A, D, E, B <sub>2</sub> , B <sub>3</sub> , B <sub>6</sub> , B <sub>12</sub> , C

The potent anti-inflammatory activity of GLM powder was confirmed *in vivo* using the established rat paw edema model; rats fed mussel lipids *per os* developed neither adjuvant-induced polyarthritis nor collagen-induced auto-allergic arthritis (CIA) (Rainsford & Whitehouse 1980, Whitehouse et al. 1997). However, these lipids showed only marginal inhibition of carrageenan-induced paw edema in rats (acute irritation assay, which is the standard test for NSAIDs), indicating that they do not mimic rapid-acting NSAIDs (Whitehouse et al. 1997). Macrides et al. (1997) found that the eicosatetraenoic acids (ETA) of GLM had considerable anti-inflammatory activity. *In vitro*, the extracted lipids have been shown to possess significant COX and LOX inhibitory activity (Whitehouse et al. 1997) (see Fig. 3, page 31). Recently, new GLM extracts were tested, and the Tween-20 extract (extracted by a cationic detergent) was noted to effectively inhibit both COX-1 and COX-2 cyclooxygenase activity (Mani & Lawson 2006). It also decreased IgG levels and induced a significant reduction in TNF- $\alpha$ , IL-1, IL-2, and IL-6, as observed using cytokine bioassays. The active components were found to possess a molecular weight above 100 kDa. GLM was suggested to mediate T-helper cell (Th1/Th2) regulation as it relates to inflammation, therefore playing an immunomodulatory role (Mani & Lawson 2006). In a recent study, they observed dose-dependent reduction in TNF- $\alpha$  and IL12-p40 production and in neutrophil superoxide burst activity, in *Perna* treated cultures (Lawson et al. 2007). In the *in vivo* part of this same study, significant amelioration of mouse CIA and significant reduction in disease incidence, onset, and severity of rat CIA was noted in the *Perna* groups of animals, compared with controls (Lawson et al. 2007).

GLM is reported to have no severe side-effects (Cleland et al. 1988, Gibson & Gibson 1998, Cho et al. 2003), but has rarely caused some fluid retention, epigastric discomfort, nausea or a transient aggravation of symptoms in some human patients (Brooks 1980, Gibson et al. 1980, 1998). In newer trials no side-effects have been reported, which might indicate that the newer products are better tolerated (Pollard et al. 2006). In fact, research suggests that GLM may have chondroprotective properties due to its GAG, especially chondroitin sulphate, content (Bassler et al. 1992, Korthauer & Torre 1992, Bucci 1994). Unlike with the use of NSAIDs, platelet aggregation is unaltered and the lipid fraction is nongastrotoxic in fasted disease-stressed arthritic rats even at a dose of 300 mg/kg (equals 15 x treatment dose) (Rainsford & Whitehouse 1980, Whitehouse et al. 1997), indicating that they inhibit predominantly the COX-2 pathway (Mani & Lawson 2006).

### 2.3.3 Homeopathy

Investigations whether, and if so, how, homeopathy works, are beyond the scope of this thesis, but some basics will be reviewed here. Two principles make a treatment homeopathic: (1) the underlying idea is to treat a disease symptom with a preparation of a natural substance, or a combination of them, that in itself could give a healthy patient the same symptoms (*similia*, the “same” principle; e.g. treating sleeplessness with coffee) (Bellavite et al. 2005, Hektoen 2005), and (2) the preparations are diluted using a certain method, potentizing, which means that they are repeatedly diluted and between every dilution the remedy is shaken vigorously. D potencies are diluted 1:10 and c-potencies, 1:100. For example, D4 is diluted 1:10 four times, and shaken 60 times between every dilution (Bellavite et al. 2005, Hektoen 2005). The final products will have an estimated molar concentration of a substance of  $10^{-1}$  mol/l, and up to  $10^{-2000}$  or more. Thus, a range of homeopathic remedies exist, where at one end dilutions actually include measurable amounts of a substance and at the other end dilutions most likely will not contain a single molecule of the substance (Hektoen 2005). Thus, the distinction between low and high-dilution effects is important where both fields fully belong to homeopathy, provided that the medicine is prescribed according to the *similia* principle and according to a holistic clinical approach (Bellavite 2006b). The very low substance concentration of the high-dilution remedies is the reason why occidental medicine has trouble understanding how homeopathy could work (Bellavite et al. 2005, Hektoen 2005). The other aspect of homeopathy that worries conventional scientists, is the claim that the more potentized, i.e. diluted, the remedy is, the more powerful are the treatment effects of it (Bellavite 2006c). Low homeopathic dilutions in the range of D1-D20 (up to 10c) may theoretically work like the extremely low concentrations ( $10^{-10}$ - $10^{-20}$  mol/l) that have been shown to be biologically active in “conventional” biochemistry and immunology (Eskinazi 1999, Bellavite 2006b).

A multitude of studies with observations that are consistent with positive homeopathical effects, have been published during the last 15 years. A multicenter study from four different laboratories in four countries has reported consistent results; high dilutions of histamine ( $10^{-30}$ - $10^{-38}$  mol/l) influence the activation of human basophils measured both by alcian blue staining and flow cytometry (Belon et al. 1999, 2004). The degree of this inhibition depended on the initial level of anti-IgE-induced stimulation, with the greatest inhibitory effect seen at lower levels of stimulation (Belon et al. 1999, 2004). Another study showed that very small amounts of hemocyanin antigen ( $10^{-36}$  M) were capable of significantly increasing a specific IgG response in mice (Wiseman et al. 1991). A carragenan-induced rat paw edema was significantly inhibited in rats treated with *Causticum* 6c, 12c, 30c, and 200c dilutions compared with untreated rats (Prado Neto et al. 2004). When the conventional anti-inflammatory

agent dexamethasone was diluted to 7c and 15c (equivalent to  $10^{-17}$  and  $10^{-33}$  mol/l), these homeopathic dexamethasones partially blocked the anti-inflammatory effect of pharmacological dose dexamethasone with regard to paw edema and polymorphonuclear cell migration into the peritoneal cavity, both significantly (Bonamin et al. 2001). A recent study showed that mice fed a daily drop of homeopathic Arsenicum Album 200 (diluted 200 times 1:100,  $10^{-400}$ ) showed significantly reduced toxicity of induced chronic arsenic poisoning in all studied parameters, compared with controls (Banerjee et al. 2007). These results suggest that a potentized substance may have both effects opposite to its own pharmacological effects, thus supporting the *similia* principle, and also, that high dilutions act on specific parameters of host response (Bonamin et al. 2001). In most of the studies mentioned above, the dilutions were lower than  $10^{-24}$ , which according to Avogadro's law should contain, on the average, at most one molecule of active principle per liter (Bellavite 2006b). Also, in all the cited studies the authors were unable to explain the method of action for these results and they also acknowledged that, in view of the vast implications of these findings, the experiments should be rigorously repeated and confirmed (Wiseman 1991).

Also, it is difficult to reject homeopathy outright since meta-analyses evaluating homeopathic studies have found significant differences between homeopathic and placebo groups, favoring homeopathy (Kleijnen et al. 1991, Linde et al. 1997, Shang et al. 2005). A complete review of homeopathy is available in Bellavite et al. (2005, 2006a,b,c,d,2007).

### **2.3.3.1 Homeopathic combination preparation (HCP) Zeel® ad us vet.**

Homotoxicology or anti-homotoxic medicine was developed from homeopathy in the 1980s in Germany (Reckeweg 1981). Although the homotoxicological remedies are somewhat different from other complex homeopathic products, they are recognized as “homeopathic remedies” by the EU drug legislation (Bellavite et al. 2006c). The term “homotoxins” is used for all substances, both exogenous and endogenous, that are harmful to man or animal. Homotoxicology uses lower dilutions (D2-D10) than those usually used in classical homeopathic remedies and are often combination, or complex, products (Heel 2000). Zeel® *ad us vet.* is a combination preparation with 14 low-dilution (D2-D8) ingredients (**Table 2**) and is used on arthropathies in e.g. horses and dogs (Boyeux 1984, Faulstich et al. 2006, Neumann et al. 2007). This product contains specific homeopathic substances as well as specific anti-homotoxic substances such as potentized suis-organ parts (homeopathically potentized organ parts from swine, representing iso-organotherapy), catalysts, and nosodes (meaning any remedy extracted from pathological tissues or microbial products) (Heel 2000, Bellavite et al. 2005).

**Table 2. Content of Zeel® ad us vet.**

The content of one 5.0 ml ampoule of the low-dilution homeopathic combination preparation (HCP): Zeel® injection solution. The third column indicates dose (mg) of the different dilutions in the ampoule, the fourth is dose of actual dry matter (DM) in one ampoule (Dil = D2-D8 = diluted 1:10 two to eight times). From the Heel Company Veterinary Guide, Baden-Baden, Germany, 1997 and from technical support.

<b>Zeel® ad us vet. 5 ml ampoule</b>	<b>Dil</b>	<b>mg dil.</b>	<b>mg DM</b>
Cartilago suis	D6	5.0	5 x10 <sup>-6</sup>
Funiculus umbilicalis suis	D6	5.0	5 x10 <sup>-6</sup>
Embryon totalis suis	D6	5.0	5 x10 <sup>-6</sup>
Placenta totalis suis	D6	5.0	5 x10 <sup>-6</sup>
<i>Solanum dulcamara</i>	D3	25.0	7.5 x10 <sup>-4</sup>
<i>Symphytum officinale e radice</i>	D6	25.0	5 x10 <sup>-7</sup>
Nadidum	D8	5.0	5 x10 <sup>-8</sup>
Coenzyme A	D8	5.0	5 x10 <sup>-8</sup>
<i>Sanguinaria canadensis</i>	D4	7.5	1.4 x10 <sup>-5</sup>
<i>Arnica montana</i>	D3	50.0	4.5 x10 <sup>-4</sup>
Sulfur	D6	9.0	9 x10 <sup>-6</sup>
Natrium diethyloxalaceticum	D8	5.0	5 x10 <sup>-8</sup>
Acidum alpha-liponicum	D8	5.0	5 x10 <sup>-8</sup>
<i>Toxicodendron quercifolium</i> <i>e summitatibus rec</i> (= <i>Rhus Toxicodendron</i> )	D2	25.0	1.2 x10 <sup>-2</sup>

Research has attempted to enlighten the mechanisms of action of different human Zeel® preparations and their constituents. In a randomized, sham-controlled placebo study on rabbits with experimentally induced knee OA, a significant difference in gross morphology and in a histopathological score was found in the joints treated with the HCP Zeel® Comp compared with untreated joints (Stancikova et al. 1999a). In an *in vitro* study, cartilage slices incubated for six days in a medium containing Zeel® showed better preservation of structure than controls, based on methods of interference polarization microscopy and x-ray diffractometry for analysis (Orlandini et al. 1997). A reconstituted Zeel® comp. N combination as well as its constituent mother tinctures showed distinct inhibitory effects on the production of leukotriene B<sub>4</sub> by 5-lipoxygenase (5-LOX) and on the

synthesis of prostaglandin PGE<sub>2</sub> by COX-1 and COX-2 enzymes (Fig. 3, page 31) (Jäggi et al. 2004). This dual inhibition of both LOX and COX metabolic pathways may offer an explanation for the reported clinical efficacy and favorable gastrointestinal tolerability of the original Zeel® comp. N remedy (Jäggi et al. 2004). An *in vitro* study (Stancikova 1999b) demonstrated that two of the ingredients in the present test product were able to inhibit leucocyte elastase activity; *Arnica* D4 up to 70% and *Rhus Toxicodendron* D3 up to 77%. In a second study, *Rhus Toxicodendron* at D1 and D2 potencies, as well as ten other plant extracts, were shown to inhibit cell growth of human cutaneous F54 fibroblasts (Valentiner et al. 2003). As a low-dilution product Zeel® *ad us vet.* contains measurable amounts of dry matter of eg. *Rhus Toxicodendron*, and may therefore be active due to working mechanisms similar to any other drug.

### 2.3.4 Acupuncture

Acupuncture is an old Oriental technique that has been used on humans in China, Japan, and Korea for at least 5000 years (Ma et al. 2005), but it is possible that it has been used as long in Europe. “Özzi”, the stone-age man that died 5200 years ago and recently was discovered in an Austrian glacier, had marks from burning the skin at acupoints, a technique still used in the orient (Dorfer et al. 1999). As Özzi was radiographed, he was found to have suffered from lumbar, knee and hip arthrosis and his colon was full of whipworm eggs. The points tattooed on his body were the same ones that one would still use to treat arthrosis and abdominal disorder. If the marked points really were used for treatment, it indicates that acupuncture was used also in Europe 5000-6000 years ago (Dorfer et al. 1999, Kothbauer 2004).

Acupuncture is currently used to treat many different diseases; in 1996 WHO listed more than 40 indications for treating humans with acupuncture but they have now reduced them to the same 13 indications that FDA lists (NIH Consensus Conference 1998, WHO 2003). Acupuncture is considered a good treatment for canine OA (Janssens 1976, Schoen 2001). The curative duration of an acupuncture needling can vary from a few hours to a number of years but must eventually be repeated to keep the patient symptom-free (Klide 1992). Treatments can be given either using traditional metal acupuncture needles or by stimulating the points by electrical current (EA), laser, injecting substances, or implanting foreign material (Altman 2001).

The acupuncture point charts available for dogs were created in the 1970s by the International Veterinary Acupuncture Society (IVAS) and are more or less directly superimposed from the human point charts (Janssens & Still 1985).

The anatomy of the acupoint permits an induced electrical current to preferentially flow through that point, meaning increased local conductivity

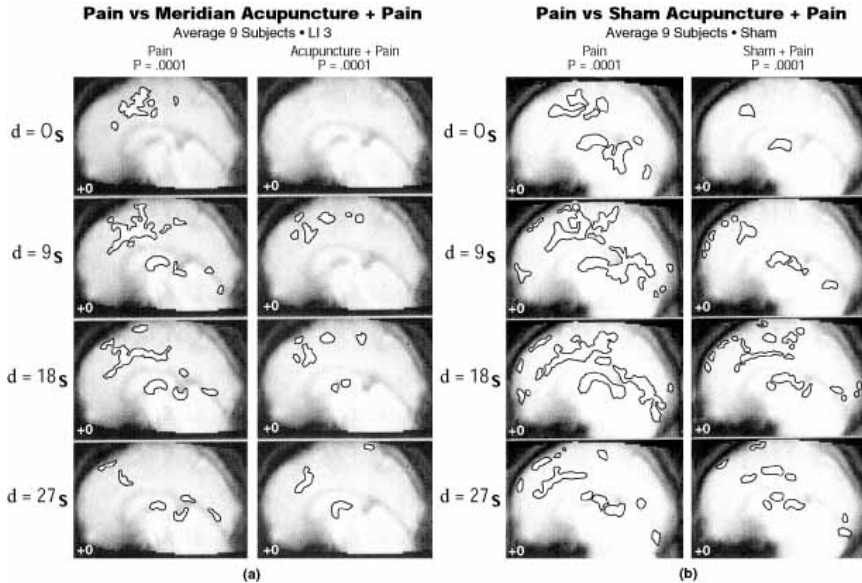
(Reichmanis 1975, 1979). A specially designed “ohm-meter” can be used to find acupuncture points as electrical searching for acupuncture points is based on the principle of the Wheatstone bridge (Burns & MacDonald 1975). The majority of acupoints have been shown to have low direct current resistance (50.000 ohms) and to be bilaterally symmetrical, compared with nonacupuncture points (200.000 – 2 million ohms) (Zhang et al. 1988, Xie et al. 1994, Pomeranz 1998). Based on anatomical findings 99.7% of acupoints are found in close proximity to peripheral nerves, 93.8% are related to superficial nerves, and 52.5% are related to deeper nerves (Zhao et al. 1993). Many acupuncture points are situated over a nerve, an artery, and a vein that travel together in a connective tissue shaft (Kothbauer 2004). Janssens et al. (1987) showed that at least some acupuncture points are not situated exactly at the same anatomical site in all individual dogs.

The mechanisms of acupuncture have been studied for over 30 years now. There are hundreds of studies and books that cover acupuncture analgesia and therefore it is covered only shortly in this text (for reviews, see Steiss 2001, Wynn et al. 2001, Cho et al. 2001, Ma et al. 2005). The six main mechanisms of acupuncture-induced analgesia are: (1) while the acupuncture needle insertion is stimulating the acute pain A-delta nerve fibers, interneurons block the C-fibers that carry chronic pain, thereby the perception of the chronic pain is not brought up to consciousness as both pain signals cannot be registered by the cortex at the same time. This is called the “pain gate theory” (Melzack & Wall 1965, Melzack & Casey 1968), (2) the acupuncture needle activates the GABA inhibitory receptors and the endogenous pain inhibitory system as well as other neurotransmitters (serotonin, noradrenaline etc.), which alter the processing of noxious information from A-delta and C fibers at various CNS levels (Pomeranz 1998), (3) the endogenous opioids (endorphins, dynorphins, enkephalins etc.) act as analgesics but are 10-200 times stronger than morphine (Pomeranz 1998), (4) segmental acupuncture analgesia is localized, with rapid onset and disappearance and not necessarily needing the higher brain centers. It is evoked by high frequency, low intensity stimulation of A-delta and C fibers, (5) there are hundreds of active endogenous substances that increase or decrease locally after introducing an acupuncture needle into an acupoint, substances (such as bradykinin, histamine, prostaglandins etc.) that have an impact on nociceptive excitation, vasodilatation, solubility, inflammation, tissue repair etc. (Kendall 1989) and (6) as pain perception now can be visualized with the help of functional magnetic resonance imaging (f-MRI): The acupuncture needle induces a reduction of brain activation in the hypothalamus and in the limbic system; bilaterally in Brodman area 24b (rostral part of anterior cingulate cortex, a key modulator of the internal emotional response to pain), ipsilaterally in 11 (orbital and basal gyri), bilaterally in the hippocampal complex and contralaterally in the amygdala formation. An increase in activity is seen in the contralateral

hypothalamus and nucleus accumbens, Broadman areas 8, 9 (prefrontal cortex) and 40 (parietal operculum) (Wu et al. 1999).

Ha (1981) demonstrated that the analgesic effect of acupuncture could be reversed by the opiate antagonist naloxone, by infiltrating the stimulated acupoint with a local anesthetic, by interruption of the dorsal part of the lateral funiculus at the upper cervical level or by ablation of the postcentral gyrus, indicating that the neural mechanisms in acupuncture analgesia take place at various levels in the nervous system (i.e. the spinal cord, thalamus, and cerebral cortex).

A recognized problem concerning placebo groups in acupuncture trials is that needling a nonacupuncture point may give the same kind of responses as needling a true acupuncture point (Debreceni 1993). There have been many studies that have researched into these mechanisms. Diffuse noxious inhibitory control (DNIC) was described in 1995 as a non-acupoint stimulus that activates the same mechanisms as acupuncture at a traditional acupoint (Helms 1995). Here activity is probably triggered in descending pathways originating from the nucleus raphe magnus (Helms 1995). The pain inhibition varies directly with the stimulus intensity; it appears to hyperstimulate a large population of A-delta and C fibers and persists after stimulation ceases (Helms 1995). It is not somatotopically organized, but triggered by a noxious stimulus from any part of the body (LeBars et al. 1979). “Hyperstimulation analgesia” is where either a strong sensory stimulus or one in the painful area can relieve pre-existing pain (Hoopwood et al. 1997), and “stress analgesia” is at least partly mediated by pituitary  $\beta$ -endorphins (Helms 1995, Pomeranz 1998). In a recent f-MRI study, sham acupuncture of nonacupoints led to a reduction of f-MRI activation in the same brain areas affected by needling a meridian acupoint (Cho et al. 2002a,b). Both stimulations reduced activation in the brain areas involved in pain perception, meridian acupuncture more than sham acupuncture but both significantly less than the pain stimulus by itself (**Fig. 4**). After these f-MRI studies, it is evident that one should not use any type of needling anywhere as a placebo (Cho et al. 2002a,b). This question of appropriate sham procedures and controls for acupuncture studies has frequently been discussed in papers by acupuncture researchers and critics alike (Ter Riet et al.1990, NIH Consensus Statement 1998, Pomeranz 1998, White & Ernst 1999, Paterson & Dieppe 2005).



**Figure 4.** Side-by-side comparison of two cortical activations seen at mid-line sagittal view, at 4 different times, during two experiments. (a) Pain stimulation alone and meridian acupuncture (at acupoint LI 3) together with pain stimulation. (b) Pain stimulation alone and sham-acupuncture together with pain stimulation. Pain stimulus was given at time  $d=\pm 0s$ .

Published with the permission of the authors and the journal (Cho et al. 2002b).

Regarding inflammation, acupuncture was indirectly shown to significantly increase T cell, macrophage, B cell and NK cell activity, but this study had no placebo group and was not blinded (Yamaguchi et al. 2007). These findings indicate that acupuncture may regulate the immune system and promote humoral and cellular activities as well as NK cell activity (Yamaguchi et al. 2007). Electroacupuncture and to a lesser degree dry needle acupuncture at an acupoint in the knee (acupoint ST36) significantly reduced CIA incidence, and IL-6, TNF- $\alpha$ , IFN- $\gamma$ , collagen II antibody, IgG, and IgM levels in CIA mouse serum, and prevented knee joint destruction (Yim et al. 2007). These results indicate that EA (and dry needling to a lesser extent) has anti-inflammatory, anti-arthritis, and immunoregulatory effects on CIA in mice (Yim et al. 2007).

#### **2.3.4.1 Gold implantation in acupuncture points of the hip**

In the early 1970s Dr Grady Young began experimenting with implanting acupuncture points in dogs to get longer lasting analgesic effects. Durkes was, however, the first to report on this method (Durkes 1989, 1992, 1994). The gold “beads” to be implanted are either manufactured gold beads or 1-mm-diameter 24-carat gold wire that is then cut into 2-mm pieces (Klitgaard 1995). The “ohm-meter” can be used to find the sites

for the gold to be implanted. Most dogs have three reactive points per hip, approximately in the same locations in different dogs. These points coincide with the three acupuncture points GB29, GB30, and BL54 (Durkes 1994, Klitsgaard 1995). Several studies without control groups have indicated success rates of 80-99% (Durkes 1989, 1994, 1999, Klitsgaard 1995, 1996, Thoresen 1996, Kothbauer 1997, Schultze 1998, 1999, 2001).

The working mechanisms of gold implants are not entirely understood. Research covering the analgesic and anti-inflammatory effects of acupuncture stimulation was reviewed above (pp. 39-41). Gold as an active material also seems to play a role (Danscher 2002). Gold, e.g. various gold-thio compounds such as injectable gold sodium, has been shown to suppress inflammation in rheumatic joints (Empire Rheumatism Council 1961). Already in the 1960s, gold was suggested to inhibit the lysosomal enzymes of phagocytotic cells in inflamed synovial tissue (Persillin & Ziff 1966). Gold ions are known to inhibit antigen processing and to suppress NF $\kappa$ B binding activity and I $\kappa$ B-kinase activation, resulting in a reduced production of pro-inflammatory cytokines (Yang et al. 1995, Traber et al. 1999, Yoshida et al. 1999). Danscher (2002) studied rats that had been implanted with 24 carat gold implants in different parts of the organism including the brain. After survival times from a few days and up to several weeks the tissue adjacent to the implants was analyzed with 1) autometallography (AMG), a histochemical technique that makes it possible to trace particles of gold only a fraction of a nanometer in diameter and with 2) particle induced x-ray emission (PIXE). He found that gold ions are released from metallic gold surfaces by a process later coined dissolucytosis (Larsen et al. 2007). This bio-release of gold ions takes place both in vivo and in vitro when macrophages are exposed to metallic gold (Danscher 2002, Larsen et al. 2007). It has been shown by AMG that in rats exposed to gold compounds such as aurothiomalate, gold ions accumulate in a multitude of cells including macrophages and cells of the proximal tubule. The gold ions can be traced ultrastructurally in lysosomes by AMG (Danscher 1981, Danscher and Stoltenberg 2006). The dissolucytotic released gold ions are taken up by the macrophages themselves and also by mast cells and fibroblasts adjacent to the gold implant. As gold ions are known to be anti-inflammatory, Danscher suggest that metallic gold implants might have an important clinical aspect. He found that the bigger the surfaces of the gold implants, the more gold ions were released and the further away gold-loaded cells could be found (Danscher 2002).

### 3. OBJECTIVES OF THE STUDIES

The primary goal of this thesis was to expand our knowledge about unconventional treatments for canine OA. To be able to evaluate treatment outcome, we also had to assess different means of evaluating chronic canine pain, as no validated pain measurements yet existed at the time of the studies.

Specific aims of the study were as follows:

1. To develop methods for assessing chronic OA pain and OA treatment outcome in dogs.
2. To test the validity and reliability of a multifactorial descriptive scale - the Helsinki chronic pain index (HCPI).
3. To evaluate the following three complementary therapies in the treatment of chronic canine OA using randomized, controlled, double-blind study designs:
  - gold implants in acupuncture points,
  - green lipped mussel *per os*,
  - homeopathic combination product *per os*.



## 4. MATERIALS AND METHODS

The following text will refer to two trials (1 and 2) and five publications (Studies I-V).

A summary of the work performed is presented in **Table 3**.

### 4.1 Dogs

Inclusion and exclusion criteria were very similar in all clinical trials (I-V). Inclusion criteria were that dogs had clinical signs and a radiographic diagnosis of OA in either a hip joint (I-V) or an elbow joint (II, IV,V). The owner had to have described at least two of the following signs as being frequent: difficulty in lying down and/or in getting up from a lying position, difficulty in jumping or refusing to jump, difficulty in walking up or down stairs, or definite lameness. Exclusion criteria were: inadequate clinical symptoms, systemic or infectious disease, neurological deficits, lameness from articular infection, or recent trauma. In trial 2, no hip-operated dogs were allowed. There was no restriction regarding uni- or bilateral OA and the lameness could be all from non-existent to severe. All dogs were otherwise determined to be in good health based on history, physical, neurological and orthopedic evaluations, and serum biochemical analysis.

Trial 1: Forty-one dogs, all of which were used in a study where different pain measurements were assessed (I) and 38 of which were included in a clinical trial where gold was implanted near the hip joint (III), randomizing them into either a treatment group (n=19) or a placebo group (n=19). Altogether 24 healthy large or giant breed dogs with no history of pain were included in trial 1 as controls for the pain questionnaire (I) and another 23 similar dogs were used as a control group for plasma hormone analyses (I).

Trial 2: Sixty-one dogs that had two baseline evaluations and that had not changed pain medication by more than one degree on the medication scale between evaluations, were used to assess an owner-evaluated chronic pain index (II). For the index responsiveness assessment, a cohort was picked after unblinding: The “medicated” group comprised NSAID-treated dogs (n = 13), and the “placebo” group (n = 11) comprised dogs that had taken

a placebo but no NSAIDs throughout the study period (II).

Altogether 44 (IV) and 45 (V) dogs finished the clinical studies of the green lipped mussel product Lyproflex (IV) and the homotoxicologic drug Zeel® (V), respectively. The dogs were divided into two treatment groups (n=15 and n=14), a positive control group (n=15), and a placebo group (n=15). All dogs had either moderate (grade D) or severe (grade E) changes in the worse affected hip joint or moderate (grade II) or severe (grade III) changes in the worse affected elbow joint (FCI 1991).

## 4.2 Study protocols

All trials (III-V) were designed as randomized, controlled, double-blind clinical trials with the help of several guidelines and recommendations (Altman 1990, Shott 1990, Budsberg 1991, Begg et al. 1996, Scheinin 1997, Farrar et al. 2000, Moher et al. 2001, Asai 2002, MacPherson et al. 2002, Turk & Dworkin 2004). All evaluators (veterinarians and owners) and technical assistants were blinded, and the dogs were assigned to groups using computer-generated random lists (III-V). For ethical reasons, all owners were also given an extra rescue NSAID (meloxicam [III] or carprofen [IV,V]) at the start of the trial to use for the dog if pain was overwhelming. No other pain treatments were allowed. The amount of rescue NSAIDs used was recorded (III-V) using the following rating: “during the last four weeks additional carprofen was given 1 = not at all, 2 = 1-2 times/4 weeks, 3 = about once a week, 4 = about 3-5 times a week, and 5 = daily/almost daily”.

Owners of all dogs were required to sign informed consent forms. The trial protocols (I-V) were approved by the Ethics Committee of the University of Helsinki.

**Table 3.** Number of dogs, examinations, samples, and types of investigations, in all studies (n= number of animals, n<sub>q</sub>= number of dogs in the questionnaire control group n<sub>b</sub> = number of dogs in the blood sample control group, N= number of investigations performed, A= performed under anesthesia).

STUDY (trial)	Dogs	Samples, examinations, treatments	N	investigation	A
<b>I (1)</b> <b>III (1)</b>	n=41 n <sub>q</sub> =23 n <sub>b</sub> = 24 n= 38	Clinical examinations	161	Rectal temperature	
			161	Palpation	
			161	Lameness exam	
			161	Jumping onto table	
			161	Climbing stairs	
			161	Hip function (ROM)	
			41	Orthopaedic examination	
			41	Neurological examination	
		Radiology	82	Official hip radiograph	x
			8	Others, when needed	x
		Plasma samples	65	Adrenaline	
			65	Noradrenaline	
			65	β-endorphin	
			65	Cortisol	
			65	Vasopressin	
		Questionnaires	595	VAS	
			287	MDS	
			123	Comparative	
		Treatment	19	Gold implants	x
			19	Sham treatment	x
<b>II (2)</b> <b>IV (2)</b> <b>V (2)</b>	n=61+7 n=45+6 n=44+7	Clinical examinations	254	Rectal temperature	
			254	Palpation	
			254	Lameness exam	
			254	Jumping onto table	
			254	Climbing stairs	
			254	Hip function (ROM)	
			254	Orthopaedic examination	
			254	Neurological examination	
		Force plate study	254	10-80 runs per dog/time	
		Radiology	68	Official hip/elbow radiograph	x
			14	Others, when needed	x
		Serum samples	254	BUN	
			254	Creatinine	
			254	ALAT	
			254	AFOS	
			254	Total protein	
			254	albumin	
		Questionnaires	508	VAS	
			254	MDS	

In trial 1, all dogs were examined at the hospital on four different occasions. At the first visit (W<sub>0</sub>), the patients were initially evaluated (I, III) and then

treated (III). Follow-up visits for reassessments were at 4, 12, and 24 weeks posttreatment ( $W_4$ ,  $W_{12}$ , and  $W_{24}$ ) (III). The hip area was clipped (depending on dog size 15 x 15 cm to 30 x 30 cm) and given a surgical scrub. All points found with the Ohm-meter (usually three) were implanted with one gold bead per site through hypodermic needles of different lengths and with a diameter of 14 G ( $\varnothing$  1.2 mm). A stiletto was used to hold the gold down in the tissue, as the hypodermic needle was retrieved. The control group was treated similarly, but no implants were inserted. Instead, they just had three “needle holes” made through the skin, using the same size needles, but with no gold and at locations that were not acupuncture points. For details of treatment, see study III. All dogs were sent home with the same instructions for rest and exercise.

In trial 2, the location of the disease (hip or elbow OA) was the only thing that was stratified for in the randomization (IV,V). The dogs were given the GLM (IV), the HCP (V) and the positive and negative control treatments (IV,V) orally for 8 weeks, from  $W_0$  to  $W_8$  (for treatment regime, see Table 4). Two control groups were included: the established positive control carprofen and a negative control, i.e. the dog received all three products as placebos. Follow-up visits with questionnaires for reassessment were at 4, 8, and 12 weeks ( $W_4$ ,  $W_8$ , and  $W_{12}$ ). At  $W_{12}$ , the dogs had been off all medication for 4 weeks and were evaluated to determine long-term effects of the different treatments.

For a time flow chart for trial 2, see Table 5, and for exact dosing, see studies IV and V.

**Table 4.** Treatment groups and medication regime used in trial 2.

Treatment groups and medication (n= number of patients per group, GLM = green lipped mussel, HCP = homeopathic combination preparation).

<b>Group (n)</b>	<b>Three products / dog, taken daily for eight weeks</b>			<b>Study</b>
<b>GLM (15)</b>	<i>Real GLM</i>	HCP placebo	carprofen placebo	<b>IV</b>
<b>Carprofen (15)</b>	GLM placebo	HCP placebo	<i>Real carprofen</i>	<b>IV,V</b>
<b>Placebo (15)</b>	GLM placebo	HCP placebo	carprofen placebo	<b>IV,V</b>
<b>HCP (14)</b>	GLM placebo	<i>Real HCP</i>	carprofen placebo	<b>V</b>

**Table 5.** Time flow chart for trial 2.

W<sub>-4</sub> - W<sub>12</sub>. W refers to week and the subscript to the number of the week before (-) or after the start of medication.

Time	Study	Procedure
W <sub>-4</sub>	II	Baseline 1, for pain assessment trial (test)
	IV-V	Any analgesic/chondroprotective medication withdrawn
		Baseline for analgesic used
	Questionnaires for owners (training)	
W <sub>0</sub>	II	Baseline 2, for pain assessment trial (retest)
	IV-V	Baseline for all other variables, force plate, veterinary evaluation
		Clinical, neurological, and orthopedic examination
		Radiographic examination
		Blood samples
		Start of medication perorally
Questionnaires for owners		
W <sub>4</sub>	II	Treatment evaluation after 4 weeks for pain assessment trial
	IV-V	Midpoint evaluation (4 weeks), force plate, veterinary evaluation
		Blood samples
	Questionnaires for owners	
W <sub>8</sub>	II	Treatment evaluation after 8 weeks for pain assessment trial
	IV-V	Endpoint evaluation (8 weeks), force plate, veterinary evaluation
		Blood samples
	Questionnaires for owners	
W <sub>12</sub>	II	Back to baseline for pain assessment trial
	IV-V	Follow-up evaluation (8 weeks), force plate, veterinary evaluation
		Blood samples
	Questionnaires for owners	

### 4.3 Assessment of chronic pain and other clinical trial outcome measures

#### 4.3.1 Owner evaluations: MDS questionnaire and VAS scales

The gathering of the “right questions” for a scale is a long pre-trial process and in the case of our study, started a year before the final first questionnaire. Face and content validity was tested as the items for the first questions were gathered from own clinical experience, previous research and literature, and from informal interviews with owners and colleagues. We finally ended up with 25 MDS questions that were tested several times until all ambiguous or poorly worded questions had been deleted or rewritten and again retested.

In our trials, the basic questionnaires consisted of different parts (I-V). The first part was an MDS questionnaire containing 25 (I,III) or 18 (II,IV,V)

questions about mood, behavior and locomotion of the dog; owners answered using a descriptive scale of 0-4 (I-V). Detailed descriptions of the questionnaires can be found in studies I and II and in **Appendix 2**. Responses 0-1 were considered typical for a healthy dog and responses 2-4 typical for a dog with chronic pain (II, IV, V).

The second part of the questionnaire consisted of two plain-line 10-cm visual analog scales (VAS): one for pain and the other for locomotion. The end of the line to the left signified no pain, or no difficulties in locomotion, whereas the right end signified the worst possible pain, or the most severe difficulties in locomotion (I-V).

In trial 1 (I,III), the MDS questionnaire and the VASs were answered seven times: at  $W_0$  as baseline before the treatment (I,III), at  $W_1$ ,  $W_2$ ,  $W_3$  at home, and at follow-up  $W_4$ ,  $W_{12}$ , and  $W_{24}$  (III). The questionnaire was entitled “Pain questionnaire for dogs with CHD”, and the first one ( $W_0$ ) was completed by the owners and veterinarians together at the hospital, and the rest of them were completed at home. From the third visit onwards (at  $W_{12}$  and  $W_{24}$ ), the owners also answered a comparative questionnaire, aimed at assessing changes in the dogs’ performance and status with respect to locomotion, climbing stairs, and signs of pain after 12 and 24 weeks. Here the owners had to choose from the following: “has improved”, “has remained unchanged”, or “has deteriorated” (III).

In trial 2 (II,IV,V), the MDS questionnaire and the VASs were answered five times:  $W_{-4}$  (II),  $W_0$ ,  $W_4$ ,  $W_8$ , and  $W_{12}$  (II,IV,V). The first one was sent home to the owners 4 weeks before the trial started ( $W_{-4}$ ) with no additional guidance as to what the different variables represented. There was no heading including the words “pain” or “assessment” on the questionnaire to avoid respondent bias (Vaillancourt 1991), the heading was simply “The general status of the dog now”. No instructions, no key to the questions, and no helpful comments were included. The owners were told that the same person would have to fill in the questionnaire every time, and to confirm this they were asked to sign each questionnaire after completing it (II). The second questionnaire was also a pre-treatment baseline questionnaire, completed just before the first visit ( $W_0$ ), before the treatments or placebos were administered (II,IV,V). Both baseline questionnaires were answered during the dry cold winter season. The three other questionnaires were given to the owners at the clinic, to be taken home and completed there, again without help or guidance (IV,V). Here, four questions about possible adverse reactions to treatment had been added, including changes in appetite, vomiting, diarrhea and atopic skin reactions. The third and fourth questionnaires ( $W_4$  and  $W_8$ ) were answered after having given the dogs the analgesic or a placebo for 4 or 8 weeks, and the last questionnaire ( $W_{12}$ ) was a follow-up, answered 4 weeks after having discontinued all treatments (IV,V). The MDS questionnaire was used both for psychometric testing (II) and to evaluate a treatments (IV,V).

The owner questionnaires were answered by owners or someone living in the same household as the dog. They had never participated in this kind of evaluation before. The questionnaires were written in Finnish language. In Trial 1 (I,III) the gold implant treatment was performed in March and in trial 2 (II,IV,V) the actual treatment period was from February to April.

#### **4.3.2 Mobility evaluations by veterinarians**

Lameness (from walk, trot, and gallop), jumping onto a small table, and climbing stairs were evaluated by two veterinarians (I,III-V). The evaluation form had a simple descriptive scale with 5-point grading, different for all three scores (**Table 6**). The three scores of both veterinarians were summed, allowing a minimum score of 0 and a maximum of 24 (2x3x4). This variable was first named “locomotion” (I,III), but in the second trial the name was changed to “veterinary-assessed mobility index” (IV,V). The latter name will now be used throughout the thesis.

In trial 1 (I,III), two veterinarians independently evaluated lameness, jumping, and climbing stairs as well as the clinical examination, including ROM testing from videotapes taken at four different times,  $W_0$ ,  $W_4$ ,  $W_{12}$ , and  $W_{24}$ . The dogs were coded by number and the videos were shown in random sequence to the evaluators. The veterinarians did not evaluate the control dogs used in the pain assessment study (I).

In trial 2 (II,IV,V), two veterinarians independently assessed mobility, as in trial 1, but in real time, i.e. no videos were used. For more details, see studies IV-V.

#### **4.3.3 Plasma hormone assays**

In trial 1, concentrations of the catecholamines adrenaline and noradrenaline,  $\beta$ -endorphin, cortisol, and vasopressin were measured. Plasma was collected from all dogs in the clinical study and from the 23 healthy blood sample control dogs (I).

In trial 2, blood samples were collected from all dogs at each visit and blood urea nitrogen (BUN), creatinine, serum alanine aminotransferase (ALAT), alkaline phosphatase (AFOS), total protein, and albumin were analyzed (IV,V).

**Table 6.** Descriptors for veterinary assessment grades.

Lameness:	0. Totally normal, no lameness 1. Slightly stiff, not so keen to move, minor lameness 2. Clearly stiff, clearly does not move freely, pacing, slightly lame 3. Clear lameness 4. Totally lame, avoids weight-bearing on affected limb
Jumping:	0. Jumps normally, well 1. A slightly careful jump 2. Jumps with a bit of difficulty, climbs up 3. Jumps or climbs with great difficulty 4. Will not even try because of difficulty/pain
Stairs:	0. Walks stairs normally 1. Slightly careful, uses both paws successively, not so keen to move 2. Sometimes uses both paws at the same time, clearly does not move freely 3. Bunny-hops all the time, walks stairs with great difficulty 4. Will not even try because of difficulty/pain

#### **4.3.4 Radiographic examination**

Radiographs were taken, at baseline (I-V) and at W<sub>24</sub> (III), of the coxofemoral joint (I-V) and/or elbow joints (IV,V), and other joints if considered relevant.

In trial 1 (I,III) dogs were sedated and positioned in ventrodorsal recumbency with limbs fully extended and the stifle joints internally rotated. An official veterinarian from the Finnish Kennel Club performed masked evaluations of all radiographs. Coxofemoral joints were evaluated for osteoarthritic changes. The degree of abnormality in a coxofemoral joint was assessed from 13 radiographic features using 2- to 5-point scales, and these were combined to form 9 variables (see **Table 7** and Study I for details). Control dogs were not radiographed.

**Table 7.** Factors evaluated from the ventrodorsal radiographs of the coxofemoral joint of dogs with CHD (I).

<b>Radiological changes</b>	<b>Evaluated as</b>
Norberg angle	>105° / 90°-105° / 75°-90° / 60°-75° / 45°-60°
Changes of femoral neck:	
-Length of femoral neck	normal / short
-Femoral neck	no exostosis / some / a lot
Area of physeal scar	no changes / some / a lot
Femoral head shape	normal / slightly flattened / very flattened / grossly deformed
Changes of acetabular rims:	
-Dorsal acetabular rim	no exostosis / some / a lot
-Cranial acetabular rim	no exostosis / some / a lot
-Caudal acetabular rim	no exostosis / some / a lot
Acetabular cavity	deep / shallow / non existent
Incongruence of joint space	no incongruence / some / total
Acetabular fossa	normal / slightly filled / totally filled
Exostosis in joint - anywhere	no exostosis / some / a lot
Bone chip in joint - anywhere	no chip / one / many

In trial 2 (IV,V), all dogs were sedated and a ventrodorsal radiographic picture of the coxofemoral and/or a lateral elbow picture was taken, only to confirm diagnosis.

#### **4.3.5 Force plate analysis**

In trial 2 (IV,V), gait was assessed by force plate analysis (Kistler force plate type 9286AA, Kistler Instrumente AG Winterhur, CH-8408, Switzerland), which objectively evaluates weight-bearing of limbs. The signal from the plate is processed and stored using a computer-based software program, and velocities and acceleration are determined by three photoelectric cells placed 1 m apart and a start-interrupt timer system (Aquire 6.0, Sharon Software Inc., DeWitt, MI, USA). The dogs were trotted from left to right by their owners. The speed had to be in the same range ( $\pm 0.5$  m/s) for the dog each time the test was performed (at  $W_0$ ,  $W_4$ ,  $W_8$ , and  $W_{12}$ ). The acceleration was  $<0.5$  m/s/s, and contact had to be made with the plate first by the forelimb and shortly thereafter by the hind limb of the same side for the evaluation to be valid. The test was repeated until sufficient valid results were obtained for both left and right limbs. A minimum of three valid measurements for each side and for each visit were then chosen by a blinded assistant, who did not otherwise participate in the study, according to speed, acceleration, and no interferences, such as gait abnormalities or extra body movements. The mean of these three measurements was used for analysis. The ground reaction forces were normalized for the body weight of each dog, and mean peak vertical force (PVF) and mean vertical impulse were used as variables. Only measurements from the most severely affected limb at time  $W_0$  were used in the analysis.

## 4.4 Statistical analyses

The number of dogs required in each group in the clinical studies (III-V) was calculated for a two-tailed test (Fisher). The sample size was designed to be sufficiently large to detect a preset difference in treatment outcome (effective vs. not effective, based on previous human or canine studies, when available) with a statistical power of 0.8 and allowing for a 5% alpha error. In all studies, all tests were two-tailed and a P-value of  $< 0.05$  was considered significant. All statistical tests were preformed using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Study I: Possible bias in the two control groups and in the group of dogs with CHD was assessed with a t-test and cross tabulation. Data obtained from the questionnaires were compared with VAS scores, with veterinarians' lameness scores, and with the radiographic changes by means of Spearman rank correlation test. Because of the uneven distribution of the data, a Mann-Whitney test was used to compare the questionnaire answers given by the owners of control dogs with those given by owners of dogs with CHD, and to compare plasma hormone concentrations in control and OA-affected dogs.

Study II: See **Appendix 2**. Construct validity was studied using principal component analysis (PCA) with varimax rotation. Only components (PC) with an eigenvalue exceeding one were interpreted (Tabachnick & Fidell 2007); in this, loadings exceeding 0.4 were emphasized (Streiner & Norman 1995). A Keiser-Mayer-Olin Measure (Kaiser 1960) of sampling adequacy that is over 0.6 shows that the data is suitable for component analysis.

Reliability of the index was assessed by two methods: 1) Cronbach's- $\alpha$  at  $W_0$  was used to check for internal consistency or the degree of mean correlation among the eleven items of the index and among items of the components extracted. 2) The test-retest reliability model was used where the mean values of the index and the eleven individual items (questions) of the questionnaire were compared at two baseline measurements (at  $W_{-4}$  and  $W_0$ ), using intra-class correlation and the Spearman correlation tests. Similarity of Cronbach- $\alpha$  at the two baseline evaluations would support a stable correlation structure. A high intra-class correlation and similarity of the mean values would indicate repeatability. Sensitivity to change of the index was studied using the independent samples t-test to compare the mean index score (and separate question) values between the medicated and placebo groups at all time points and especially during medication, i.e. at  $W_4$  and  $W_8$ . Provided that to the two groups do not differ before ( $W_{-4}$  and  $W_0$ ) medication, smaller scores (meaning less pain) in the medicated group during medication would indicate the index's sensitivity to change. Baseline bias between the treated and placebo group was assessed with a

chi square test and cross tabulation. At  $W_{-4}$ ,  $W_0$  and  $W_{12}$ , neither of the groups were medicated; they are thus expected not to differ with respect to the index score and even individual item means. Testing of this expectation calls for similarity testing (Schuirmann 1987) that fundamentally differs from the ordinary use of the standard t-test. In its strictest sense, similarity testing requires a pre-statement of a similarity limit (Schuirmann 1987). Because the maximal clinically tolerated difference in evaluated pain is not known, we relied on the PDS (potentially data supported) approach of Rita and Ekholm (2007): it gives the largest difference of the index score (and item) means that is statistically consistent (at e.g. 0.05 risk level) with the observations. Hence, if the obtained PDS-difference can be regarded as clinically small, we gain statistical support to the similarity of the index score and item means of the two groups at the time points when their medication is the same. The same PDS-approach was also applied to argue for similarity of correlations and differences of the two baseline measurements at  $W_{-4}$  and  $W_0$ .

Study III: Bias for major background variables between the two groups was calculated using Chi-square tests. The Sign test was used to evaluate the improvement in locomotion seen by the veterinarians. There, if the grades for the five variables concerning mobility had either all improved or some improved and some stayed unaltered, the mobility index was considered to have “improved”. If the variables had either all deteriorated or some deteriorated and some stayed unaltered, then the mobility index was considered to have “deteriorated”. If there was no change in any variable or if there were both changes for the better and changes for the worse, they were “unchanged”.

The Spearman correlation test was used to test the assessments of the two veterinarians. Analysis of variance for repeated measures, with time as a within-factor and treatment group as a between-factor, was used to study the temporal patterns of the owner reported VAS data.

Studies IV and V: For calculating the percentage of dogs per group that improved between baseline and  $W_8$ , the results of each variable were converted into dichotomous responses of “improved” and “not improved”. Dogs that deteriorated, that used rescue carprofen more than three times per week at  $W_8$ , and dogs with no change in the evaluated variable were considered “not improved”. Differences between the treatment groups and the two control groups were calculated using a Chi-square test. The odds ratio was calculated using the common Mantel Haenszel odds ratio estimate, and the confidence interval (CI) was set to 95%. An odds ratio (including CI) over 1.0 indicated a beneficial effect of the tested treatments.

The change from baseline to  $W_8$  was also calculated as a mean and median for each variable. For dogs that had used extra carprofen more

than three times per week at  $W_8$ , the variable values at evaluation  $W_8$  were changed into the most negative value measured at that time, separately for each variable. This was done to counteract the effect of the extra NSAID taken and enabled us to use the whole data in the statistical analyses. The difference between the two tested treatments and the two control groups were analyzed using the Mann-Whitney U-test. The change from  $W_0$  to  $W_8$  in the force plate variables was similar for the front and hind limbs, although the values were different. Therefore, force plate data collected from all four limbs were analyzed together.

Dogs for which we did not manage to get force plate results due to major lameness were considered “not improved” in the dichotomous analyses, but were excluded from the median change data. A Pearson correlation test was used to evaluate the association between the assessments of the two veterinarians.

## 5. RESULTS

All evaluations of outcome and pain assessing variables were done during trial 1 (I). Further validation of a MDS questionnaire was done during trial 2 (II). Some of the variables were used as outcome measures in the clinical studies (III-V).

As no statistically significant bias existed between the groups at baseline in any of the trials, no adjustments were necessary.

### 5.1 Pain/outcome assessing studies (I-II):

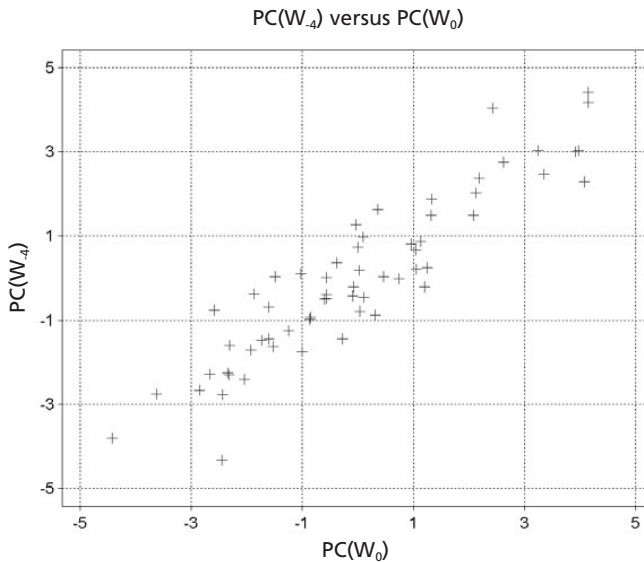
#### 5.1.1 Owner evaluations (I,II)

##### ***5.1.1.1 MDS questionnaire and the Helsinki chronic pain index (HCPI) (I,II)***

**Validity (I) – In trial 1 (I)** comparison of the questionnaire answers provided by owners of dogs with CHD with those provided by owners of control dogs with no pain revealed significant differences ( $P < 0.001$ ) in answers to 17 of the 25 questions (Table 1 [I]). Eleven of these 17 were selected, as “bad” items that either were not applicable to all owners (e.g. stair climbing), that were not easily understood (e.g. pacing) or that did not show a significant difference between healthy and diseased dogs (e.g. appetite), were dropped at this stage, resulting in the eleven item Helsinki chronic pain index (HCPI) (see **Appendix 2**). The index number was derived from the 5 possible answers (scores of 0-4); for the 11 questions selected, there was a possible minimum index number of 0 (11 x 0) and a possible maximum index number of 44 (11 x 4). There was a significant difference in the HCPI between the CHD dogs and the used control dogs of our first study; index range 7-35 and median 19 compared to range 0-5 and median 2, respectively (Table 1 [I]). The dogs that were given rescue analgesics were scored as having shown significantly more pain (veterinarians  $P = 0.006$ , owners  $P = 0.016$ ) than the dogs not given meloxicam, already indicating that the first questionnaire was sensitive. The HCPI correlated significantly with the pain and locomotion VAS scores for dogs with CHD ( $R = 0.65$ ,  $P < 0.001$  and  $R = 0.65$ ,  $P < 0.001$ ) (Table 2 [I]).

**In trial 2 (II)**, the cohort of 61 dogs with OA had a chronic pain index of 4-28 (median 17). The Kaiser-Meyer-Olin Measure (Kaiser 1960) of sampling adequacy was equal to 0.78, showing that the data is suitable for component analysis. Communality values tended to be moderate to good

(0.30-0.86). The unrotated PCA component matrix at  $W_{-4}$  and  $W_0$  extracted three components with an eigenvalue  $>1$ , but the scree plot suggested only one component (PC) should be accepted (Figure 1 [II]). The accepted first component's 10/11 items had very similar loadings at  $W_{-4}$  and  $W_0$ , (0.44 to 0.68 and 0.44 to 0.76, respectively), vocalization being the only item with a loading of only 0.20 and 0.27, respectively (see Figure 2 [II]). There was a high correlation at  $W_0$  between the PC( $W_0$ ) and the HCPI value,  $r=0.99$ . The mutual correlation of PC( $W_{-4}$ ) and PC( $W_0$ ) was high (0.91) (Fig. 5).



**Fig. 5.** Scatterplot of the mutual correlation of PCs (all 11 items) at  $W_{-4}$  and  $W_0$  ( $R=0.91$ )

When further evaluating construct validity, a varimax rotation at  $W_{-4}$  extracted three components with an eigenvalue over one that we were not able to interpret while the rotation at  $W_0$  extracted three components that could easily be clinically interpreted. These three components (PC1,-2,-3) at  $W_0$  explained 59.1 % of the total variation among the eleven index items. In the first PC1( $W_0$ ) there were eight items (questions 4 to 11) with higher component loading (0.50-0.80) and as they were all related to mobility, we called this component “mobility”. The second component PC2( $W_0$ ) had two items related to “mood” (questions 1 and 2) with component loadings of 0.78-0.92. The third component PC3( $W_0$ ) had one single item, “vocalization” (question 3) with a loading of 0.88 (to see the items, see **Appendix 2**).

Internal consistency (II) – The Cronbach’s- $\alpha$  of the eleven questions at time  $W_0$  was 0.82, with an inter-item correlation mean of 0.31, indicating internal consistency through an acceptable level of mean correlation among the

questions in the questionnaire. If single items were deleted, the Cronbach- $\alpha$  values still ranged from 0.79 to 0.83. The fact that the reliability coefficients of the index here remain high, emphasize the internal consistency reliability of the index. If the index was looked at as having three components, the Cronbach's  $\alpha$  of the two first components at time  $W_0$  were  $PC1(W_0)=0.81$  and  $PC2(W_0)=0.80$ , whereas  $PC3(W_0)$  could not be calculated since the component included only one item.

Repeatability (II) –The 11 items (and the total HCPI score) had an intra-class correlation of 0.90 when tested at the two baseline evaluations four weeks apart (at  $W_{-4}$  and  $W_0$ ), indicating a high test-retest reliability. The Pearson rank correlation between the HCPI index scores measured at the two baselines, four weeks apart (at  $W_{-4}$  and  $W_0$ ), was 0.92 ( $P<0.001$ ) and between two same items the Spearman correlation coefficients ranged from 0.62 to 0.88. The PDS was calculated to ensure reliable results, despite small sample size. The smallest PDS correlation (Rita & Ekholm 2007) that was consistent with the HCPI score was 0.82 (Table 1 [II]).

At the two baselines, the mean HCPI total scores were similar: the observed mean HCPI total score decrease from  $W_{-4}$  to  $W_0$  was only 0.278, which is less than 2% of the mean index value at  $W_{-4}$  (16.00). Calculated using PDS, the largest consistent population change (0.752) would be around 4.7%. The individual items naturally showed more variation. The largest PDS calculated consistent population change would be, however, 10% at most.

Sensitivity to change (II) –There was no significant baseline bias in HCPI total score, rescue medication, gender, age, bodyweight or time having suffered from OA symptoms, between the two treatment groups. The HCPI total score means in the placebo and medicated group were similar at  $W_{-4}$  and  $W_0$ , their differences (1.87 and 0.79, respectively; Table 2 [II]) being 11 and 5% of the corresponding index mean in the placebo group (17.20 and 16.64).

During medication, the index mean in the placebo group exceeded that of the medicated group by more than 60% at  $W_4$  and by 123% at  $W_8$  (HCPI of medicated group during  $W_4$  and  $W_8$  were 9.62 and 7.69, respectively) (Table 2 [II]), indicating that the index is sensitive.

#### **5.1.1.2 VAS scales (I)**

In trial 1 (I) the pain and locomotion VAS scores for dogs with CHD correlated significantly with each other ( $R=0.71$ ,  $P<0.001$ ). Neither of them correlated with the veterinarians' combined locomotion score ( $R=0.06$ ,  $P=0.72$  and  $R=0.17$ ,  $P=0.29$ , respectively).

### **5.1.2 Veterinary-assessed mobility index / locomotion (I)**

In trial 1 (I) the locomotion evaluations provided by the two veterinarians were in agreement (lameness,  $R=0.60$ ,  $P\leq 0.001$ ; ability to jump on and off a table,  $R=0.78$ ,  $P<0.001$ ; and ability to climb and descend stairs,  $R=0.63$ ,  $P\leq 0.001$ ). Some correlation between the calculated HCPI and the veterinarians' combined score was detected ( $R=0.35$ ,  $P=0.047$ ), but there was poor correlation between most of the answers to individual questions (Table 2 [I]).

### **5.1.3 Plasma hormone assays (I)**

A significant increase in adrenaline, cortisol, and vasopressin and a significant decrease in  $\beta$ -endorphin concentrations was seen between the controls and the dogs with CHD (Table 3 [I]). There was considerable individual variation in all of the measured plasma hormone concentrations. Adrenaline concentration had a significant positive correlation with noradrenaline and cortisol concentrations. Neither of the two VAS scores nor the veterinarians' combined score correlated with plasma hormone concentrations in dogs with CHD.

### **5.1.4 Radiographic examination (I)**

A significant correlation was found between the Norberg angle and change within the area of the physal scar ( $R=0.52$ ,  $P=0.001$ ), shape of the femoral head ( $R=0.44$ ,  $P=0.009$ ), exostosis of the acetabular rims ( $R=0.35$ ,  $P=0.034$ ), the acetabulum ( $R=0.72$ ,  $P<0.001$ ), and appearance in the acetabular fossa ( $R=0.65$ ,  $P<0.001$ ). Of the radiographic variables, none correlated with the veterinarians' combined mobility score or with the owners' pain VAS score. Variables that correlated with the owners' locomotion VAS score were exostosis in any region of the joint ( $R=0.34$ ,  $P=0.04$ ) and number of bone chips in any region of the joint ( $R=0.34$ ,  $P=0.034$ ). No correlation existed between duration of clinical signs of CHD and severity of radiographic abnormalities.

## **5.2 Experimental treatment studies (III-V):**

### **5.2.1 Gold implant study (III)**

#### **5.2.1.1 Owner evaluations (III)**

The VAS data submitted by the owners showed overall a highly significant improvement in the treatment group in locomotion and a highly significant decrease in pain ( $P=0.0001$  and  $P=0.0034$ ) during the trial period (for locomotion, see Fig. 4 [III]). These differences were not statistically significant between the two treatment groups ( $P=0.41$  and  $P=0.24$ , respectively). When changing outcome to "improved", "unchanged", or

“deteriorated”, 53% of treated dogs and 63% of control dogs were evaluated as improved, but the differences between the treated and control groups were not statistically significant ( $P=0.80$ ) (Fig. 3 [III]).

When the blinded owners were asked to evaluate the success of treatment at the end of the study, no significant difference emerged between the two treatment groups ( $P=0.895$ ). In the comparative questionnaire, there were no statistically significant differences in tested variables between groups at either  $W_{12}$  or  $W_{24}$ .

#### **5.2.1.2 Veterinary-assessed mobility index/locomotion (III)**

When comparing  $W_{24}$  with  $W_0$ , a significant improvement was found in the veterinary-assessed mobility index for the treated group ( $P=0.036$ ). However, the mobility index showed no statistically significant difference between the two groups ( $P=0.19$ ). According to the blinded veterinarians, 65% of treated dogs and 53% of control dogs had improved locomotion (Fig. 2 [III]). When comparing the two veterinarians' assessments of hip function, there was a very low negative, non-significant correlation ( $R=-0.15$ ), so these results were not reported.

#### **5.2.1.3 Radiographic examination (III)**

No migration of implants was detected in radiographs of the coxofemoral joint taken at  $W_{24}$ , six months after implantation.

#### **5.2.1.4 Complications and side-effects (III)**

No side-effects were reported. Two dogs from the placebo group were euthanized between weeks 12 and 24 because of signs of intense pain due to OA.

### **5.2.2 GLM and HCP studies (IV,V)**

Baseline values for individual groups in studies IV and V can be seen in **Table 8**. No statistically significant bias existed between the groups at baseline in any of the trials.

Eight of the 68 dogs were excluded from the material at some time during the study because they no longer met the medical inclusion criteria (operation on the affected hip joint ( $n=1$ ), transverse vertebra diagnosed after inclusion ( $n=2$ ), cruciate ligament injury ( $n=2$ ), degenerative myelopathy ( $n=1$ ), polyarthritis of the phalanges ( $n=1$ ), and castration just prior to the third visit ( $n=1$ )). There were 4 dogs, all from the placebo group, that had used extra carprofen more than 3 times per week at  $W_8$ , and two dogs from the placebo group and one from the GLM group were unable to trot over the force plate (see Statistical Analyses, p. 54). The odds ratios for all variables are given in studies IV and V.

**Table 8.** Distribution of possible confounding factors between groups at time  $W_0$  (for extra NSAIDs at  $W_{-4}$ ) (GLM = green lipped mussel, HCP = homeopathic combination preparation, NSAID = nonsteroidal anti-inflammatory drug, OA = osteoarthritis, SD= standard deviation, PVF = peak vertical force, VAS = visual analog scale) (IV,V).

Possible confounding factors	Carprofen	GLM	HCP	Placebo
n	15	15	14	15
n with hip dysplasia	12	12	12	12
n with elbow OA	3	3	2	3
Sex: Male/Female	7/8	8/7	8/7	10/5
Median age (years); Min – Max	5 1 - 9	7 2 - 10	7.5 1 - 11	6 1 - 11
Median duration of signs (years)	> 2	1-2	1-2	1-2
Median weight (kg); Min – Max	38 31 - 56	34 20 - 49	27.5 22 - 54	34 18 - 54
<b>Mean ± SD of continuous variables at start of trial</b>				
Veterinary-assessed mobility index	5.00 ± 4.61	6.07 ± 4.51	6.79 ± 6.46	5.20 ± 4.26
Force plate-PVF	75.92 ± 23.48	77.41 ± 16.69	70.96 ± 22.58	78.46 ± 22.23
Force plate-impulse	10.92 ± 4.02	10.07 ± 3.04	8.64 ± 3.06	9.72 ± 3.43
Owner-assessed Chronic pain index	16.47 ± 6.21	14.60 ± 4.76	15.86 ± 6.20	14.87 ± 4.79
Owner-assessed pain VAS (cm)	3.55 ± 2.17	3.87 ± 1.82	4.24 ± 2.16	3.70 ± 1.77
Owner-assessed locomotion VAS (cm)	4.57 ± 2.03	4.36 ± 2.03	4.87 ± 2.26	4.61 ± 2.12
<b>Median, Min - Max of variable at 4 weeks prior to trial (<math>W_{-4}</math>)</b>				
NSAID doses per month	none, none - 3-5/week	none, none – about 1/ week	none, none – daily/ almost daily	none, none – about 1/ week

### 5.2.2.1 Owner evaluations (IV,V)

When the data of all variables had been converted to the dichotomous responses of either “improved” or “not improved”, there were significantly more improved dogs in the GLM group than in the placebo group according to two of the three owner-assessed variables; HCPI (P=0.028) and pain VAS (P=0.011). Locomotion VAS was not significant, but showed a similar trend (P=0.070) (**Table 9**).

Using the same dichotomous responses for the HCP study (V), only the pain VAS (P=0.043) of the three owner-assessed variables indicated significantly more improved dogs in the HCP group than in the placebo group (**Table 9**).

A highly significant difference was present in the extent of improvement between the GLM and placebo groups in the pain VAS ( $P=0.004$ ), and a second variable was close to being significant; locomotion VAS ( $P=0.057$ ). The difference in the extent of improvement between the HCP and the placebo was significant according to the chronic pain index ( $P=0.049$ ) and the pain VAS ( $P=0.020$ ) (**Table 9**).

#### **5.2.2.2 Veterinary-assessed mobility index (IV,V)**

When the data of all variables were converted to the dichotomous responses of either “improved” or “not improved”, according to the veterinary-assessed mobility index there were significantly more improved dogs in both the GLM group ( $P=0.031$ ) (IV) and the HCP group ( $P=0.018$ ) (V) than in the placebo group (**Table 9**).

There was also a significant difference in the extent of improvement in the veterinary-assessed mobility index between both GLM ( $P=0.012$ ) and HCP ( $P=0.015$ ) groups and the placebo group (**Table 9**). The evaluations of the two veterinarians correlated well ( $R=0.853$ ,  $P<0.01$ ) in both studies (IV,V).

**Table 9.** Percentage of improved dogs and median (range) of improvement for evaluated variables, per group from  $W_0$  to  $W_8$ . For each treatment group: First column: Percentage of dogs in the group that improved. Second column: Median (with range) of change from  $W_0$  to  $W_8$  ((+) = improvement, (-) = deterioration) in evaluated variables for the carprofen, GLM and placebo groups. P = Difference in improvement between treatment groups and placebo (the force plate values do not include three dogs for whom no results were obtained). (n = number of patients per group, GLM = green lipped mussel, PVF = peak vertical force, VAS = visual analog scale)

	Carprofen (n=15)		GLM (n=15)		HCP (n=14)		Placebo (n=15)	
	Improved % P=	Improvement Median (range) P=	Improved % P=	Improvement Median (range) P=	Improved % P=	Improvement Median (range) P=	Improved %	Improvement Median (range)
Owner: Chronic pain index HCPI	80.0 % 0.028	9 (-9-19) <0.001	80.0 % 0.028	2 (-2-6) 0.102	57.1 % 0.364	2 (-6-9) 0.049	40.0 %	-3 (-25-8)
Owner: Pain VAS	85.7 % 0.001	1.4 (-6-8.4) <0.001	66.7 % 0.011	0.6 (-3.3-3.3) 0.004	57.1 % 0.043	0.2 (-3.5-4.9) 0.020	20.0 %	-1.7 (-7-3.2)
Owner: Locomotion VAS	85.7 % 0.002	3.1(-1.9-6.2) 0.001	60.0 % 0.070	0.2 (-3.8-3.5) 0.057	57.1 % 0.102	0.7 (-5-4.8) 0.205	26.7 %	-1 (-6.6-5)
Veterinary mobility index	66.7 % 0.031	3 (0-8) 0.001	66.7 % 0.031	1 (-3-7) 0.012	71.4 % 0.018	1.5 (-5-7) 0.015	26.7 %	-3 (-14-3)
Force plate PVF	66.7 % 0.031	3.2 (-8.2-11.8) 0.079	46.7 % 0.264	0.17 (-5.6-12) n=14 0.201	78.6 % 0.006	2.3 (-3.4-10.2) 0.028	26.7 %	-0.9 (-33.6-10) n=13
Force plate impulse	80.0 % 0.011	0.4 (-0.5-1.3) 0.009	53.3 % 0.277	0.20 (-1-1.54) n=14 0.123	64.3 % 0.101	0.2 (-1.3-1.3) 0.093	33.3 %	-0.0 (-3.3-0.8) n=13

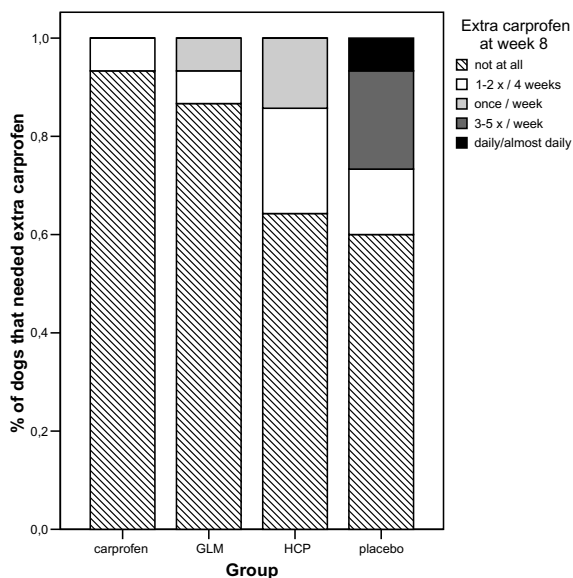
### 8.3.6.1 Force plate analysis (IV,V)

None of the force plate variables indicated significant differences between the GLM product and the placebo (Table 9).

In the HCP study, when the data of all variables were transformed into dichotomous responses of either “improved” or “not improved”, the PVF was significantly better in the HCP group ( $P=0.006$ ) than in the placebo group. A significant difference was also present in the extent of improvement in the PVF ( $P=0.028$ ) between the HCP group and the placebo group (Table 9).

### 5.2.2.3 Intake of rescue NSAIDs (IV,V)

At  $W_{-4}$ , before the owners were told to stop all medication, 14% of dogs in the carprofen group, 13% in the GLM group, 28% in the HCP group, and 8% in the placebo group were given rescue NSAIDs once a week or more. At  $W_8$ , 0%, 7%, 14%, and 27% of the respective groups were given additional carprofen once a week or more (Fig. 6). At follow-up ( $W_{12}$ ), the respective numbers were 33%, 14%, 21%, and 29%. In the GLM study (IV), the differences between both GLM and carprofen compared with the placebo group at time  $W_8$  were significant ( $P=0.021$  and  $P=0.008$ , respectively), but in the HCP study (V), only the difference between the carprofen group and the placebo group was significant ( $P=0.012$ ) at  $W_8$ .



**Fig. 6.** Proportion of dogs given extra rescue NSAIDs (Carprofen) at  $W_8$  in all 4 treatment groups. Columns indicate the percentage of dogs per group that were administered extra carprofen during the 4 last weeks according to the following scale: 1 = not at all, 2 = 1-2 times, 3 = about once a week, 4 = about 3-5 times a week, and 5 = daily/ almost daily.

### 5.2.2.4 Comparing the tested treatments to carprofen (IV,V)

When the data of all variables were converted to the dichotomous responses of either “improved” or “not improved”, none of the variables in

either study showed a statistical difference between tested product and the positive control group, given carprofen.

There was a significant difference in the extent of improvement only in the HCPI and in the locomotion VAS, but between both the GLM (P=0.004 and P=0.005) and HCP (P=0.007 and P=0.019) groups and the carprofen group, where carprofen was more effective.

#### ***5.2.2.5 Complications and side-effects (IV,V)***

In the study populations, all of the altered blood values and the clinical side-effects were considered mild or within normal range (IV,V). Two of the dogs for which no data were achieved on the force plate (one in the GLM group, one in the placebo group) were euthanized between W<sub>8</sub> and W<sub>12</sub> due to severe OA pain.

## 6. DISCUSSION

### 6.1 Dogs

Dogs with CHD were selected as the target population since dogs with this disease form a typical group of patients suffering from chronic OA and due to that, pain. Also, they were considered to be readily available. The dogs used are representative of a normal population of canine OA patients; many dog breeds were included, but more of the breeds with higher incidence of CHD or ED were represented (statistics of the Finnish Kennel Club). The two trials were not totally comparable. The first trial comprised dogs suffering from CHD (I,III). However, as we had difficulty in getting enough dogs with CHD for the second trial, also dogs with OA due to ED were included (II, IV,V). To minimize bias, the dogs suffering from OA due to ED were stratified when allocated into groups. The problems with heterogeneity in the cohort will be discussed in the clinical trial section.

The placebo and carprofen have in previous studies shown 23-38% and 56-81% of improvement in dogs with OA, as graded by veterinarians and owners (Holtsinger et al. 1992, Vasseur et al. 1995). As these numbers are similar to the results for these two groups in our second trial (26-40% and 66-86%), our cohort appeared to reflect reality well.

### 6.2 Variables chosen for outcome assessment

The results of our clinical trials highlight a problem with outcome assessment. In the second trial, we used 6-7 variables to assess treatment outcome. Depending on which of these are chosen as primary outcome measures, the results can vary markedly. According to force plate PVE, the homeopathic drug was very good, yielding 88% of the improvement produced by carprofen, while the placebo deteriorated 19%. The second force plate variable, vertical impulse, or locomotion VAS, indicated that GLM and HCP were of no use, with no significance being found for the two variables in either number of dogs improved per group or rate of improvement. According to the pain VAS, both treatments were effective, but no references to its validation as a valid outcome measure can be found in the literature. We have shown that the HCPI index is a good measure, but according to this outcome measure, only one of the two aspects is significant

for each of the two tested treatments. As seen, outcome assessment is not straightforward. Efforts should therefore continue to be directed toward finding valid and reliable chronic pain outcome measures to be used in research, as was done in our first two studies (I,II).

### **6.2.1 Owner evaluations**

At the time of our first study, some articles had already been published on acute and postoperative pain evaluated by professionals such as veterinarians or technicians (Conzemius et al. 1997, Firth & Haldane 1999). As there were no validating studies on canine chronic pain scales and because we needed chronic pain outcome measures to evaluate our treatments in the clinical trials, we decided to look into this. Innes and Barr (1998) were to our knowledge the first to publish an owner used VAS tool to assess outcome after an orthopedic procedure for dogs. The reliability and responsiveness were estimated and found to be acceptable. As in our study, they found only few correlations between the different VAS scales and veterinarian assessment. Some years later Reid et al. (2000) published an abstract on the same topic and they continued their interest in this issue and have now developed a structured questionnaire on the basis of effects on health-related quality of life (Wiseman-Orr et al. 2004, 2006). Their GUVQuest questionnaire is, however, very different from ours. Their questionnaire was developed from 109 chronic pain-describing items in 13 factors that were tested on 182 owners of dogs suffering from chronic pain. While our HCPI has 9 of 11 variables that have to do with mobility, of their 13 factors, only 1 comes from mobility. Our index may therefore be more appropriate for dogs suffering from chronic pain due to an orthopedic problem, while the GUVQuest might prove itself useful also for other causes of chronic pain. While our HCPI is now thoroughly tested (II), their scale is validated, but still awaits reliability testing.

A third questionnaire for use in assessing pain and lameness in dogs was later validated and tested for repeatability (Hudson et al. 2004). This scale started off with 39 questions that were presented as VAS scores, but none of them was an original pain VAS with endpoints from “no pain” to “most possible pain”. These authors ended up with 11 valid and reliable questions about locomotion, demeanor and mood (Hudson et al. 2004). They correlated the results of the questionnaire with a force plate evaluation. This would have been possible for us to do as well, but since our force plate measurements were so variable we opted not to. Of the 11 questions in Hudson et al. (2004), five to six are similar to the 11 questions in our HCPI (I,II) and seven of them deal with different aspects of mobility. As the HCPI and their VAS questionnaire are quite similar in content, but different in design, only time will show which of these two will be more used.

Very recently a fourth chronic pain outcome measure has been introduced; the Canine Brief Pain Inventory (Brown et al. 2007). This

instrument has 10 items with a 11 point rating scale and one “Quality of life” question with a five point categoric response. Compared with our single factor scale, it has two factors: “severity of pain” and “interference with function”. This instrument was thoroughly tested and found to be valid and reliable.

Different researchers have used different ways of showing validity and reliability. For our index, reliability was shown with an intra-class correlation of 0.9, indicating a high test-retest reliability. A measure as small as 0.50 is considered sufficient for adequate temporal reliability for questionnaires (Cohen 1988, Streiner & Norman 1995). Reliability of the index structure in time can also be tested by comparing the Cronbach’s- $\alpha$  at two basically similar evaluations. As the rank correlations between the index values at the two baseline evaluations for our HCPI was high, this also indicates that the chronic pain index is reliable. For the same reason the individual questions per se can also be regarded as reliable (Table 1 [II]). Our individual question correlations of  $r=0.62-0.88$  were very similar to the correlations of Hudson et al. (2004),  $r=0.68-0.90$ . Brown et al. (2007), showed a high test-retest reliability with  $\kappa$  values of 0.75 and 0.81 for their two factors. Wiseman-Orr et al. (2006) have not yet tested their GUVQuest for repeatability.

Internal consistency was measured by component analysis, showing that at least 10 of the 11 questions were measuring the same thing and that the odd item (vocalization) was constant from time to time, but not a typical pain sign for all dogs. The individual components of the index gave rise to one or three interpretable principal components, but the one component model was more suitable according to psychometric testing (Kaiser 1960, Cattell 1978, Tabachnik & Fidell 2007). The one component model seemed to indicate a general level of pain, as the loadings of individual questions were very similar; except for vocalization. This is further supported by their high correlations with the index values. As the mutual correlation of the PCs at  $W_{-4}$  and  $W_0$  was high (Fig. 5, page 58) and the loadings at these times were very similar (Fig. 2 [II]), the index structure shows no trend in time, indicating internal consistency. A mean inter-item correlation  $>0.3$  is considered good for an eleven item questionnaire (Nunnally 1978, Carmines & Zeller 1979).

Sensitivity to change (also called responsiveness) was shown as owners in the treatment group recognized a clearly better or more positive mood, behavior and locomotion of their dog when dogs were given daily analgesic medication compared with those that received no such medication. Previous studies have demonstrated the analgesic effect of carprofen in dogs with chronic pain (Holtsinger et al. 1992, Vasseur et al. 1995, Borer et al. 2003), and the results of our study support these conclusions. None of the other studies have yet tested their questionnaire for sensitivity to change.

The HCPI has now been thoroughly tested and is considered suitable when dog owners should assess clinical outcome in a chronic pain trial. However, the HCPI has still only been evaluated for dogs with chronic pain due to OA and could also be evaluated for chronic pain due to other causes. As a change in language may have an impact on results, it should also be tested in English before one can be sure that the results of the psychometric tests are generally valid. It would also be interesting to determine cut-off points for severe, moderate, and mild pain in order to be able to better categorize pain for research purposes, but it is possible that it will not be possible with this index, since vocalization is a sign that not all dogs with chronic pain will show. It might prove difficult if one, based on the index, would try to distinguish dogs in pain from healthy dogs or try to find cut-off points to assess when analgesic medication should be given. In our first study (I) the items of our index were tested for construct validity by extreme groups (Streiner & Norman 1995), where dogs suffering from pain due to OA were compared to healthy dogs, having no pain. Only Brown et al. (2007) have validated their scale in a similar way. Neither of the other studies presented above mentioned mean or median values for the different questions, cut-off points or any of these other factors to be looked into (Wiseman-Orr et al. 2004, 2006, Hudson et al. 2004).

Potentially data supported results can be used to back up results from a smaller cohort. As many psychometric tests recommend the use of 100-300 evaluators when testing a scale, our cohorts can be regarded as small. To compensate for this, we have paid special attention to the quantitative features in the analysis by also reporting the results as potentially data supported (PDS) values (Rita & Ekholm 2007). The PDS-approach enables estimation of the worst situation in a corresponding population (i.e. a population of similar dogs suffering from chronic pain due to OA) that is statistically consistent with the data. The approach gives us a value of the lowest possible significant correlation (at  $p < 0.05$ ) and the largest possible significant difference (at  $p < 0.05$ ) for a hypothetical larger, similar, cohort.

The strength of our HCPI index is that it is not too long to be overwhelming for the owner, nor too short to have a weak reliability. Made up of 11 separate questions, it has shown that it is capable of detecting a change in pain experience even in very small groups of dogs, although all questions per se do not show a significant difference between treated and non-treated dogs.

The pain and locomotion VASs are not discussed in this thesis as they require further attention and will be dealt with in two new articles later (in process). In a review concerning childrens pain it was concluded that the observational pain VAS had not yet been sufficiently tested, so that one could be sure of its adequate sensitivity (van Dijk et al. 2002).

### **6.2.2 Mobility evaluation by veterinarians**

In trial 1, a blinded veterinary evaluation from film material was used. Although lameness could easily be evaluated, the two veterinarians found it very difficult to evaluate hip function and pain from video tapes. Since this resulted in a poor correlation between evaluating veterinarians in Study III, indicating poor inter-rater reliability, it was not used in our second trial. The evaluated mobility had a good inter-rater reliability indicating that lameness evaluations can be done from videos. A surprising finding was that the veterinary mobility index did not correlate at all with the owner evaluated locomotion VAS although both should have been measuring the same thing.

In trial 2, we used two blinded veterinarians who assessed the dogs at the hospital (IV,V), which is one of the most commonly used outcome measures in clinical trials. It is striking that there seems to be no validated evaluations for veterinarian use. Although many different types of lameness scores, palpation scores, ROM scores, etc., have been used, none of these has, to our knowledge, been tested for validity or reliability. This was criticized in some recent articles (Schulz et al. 2006, Brown 2007, Kapatkin 2007a), as rigorous evidence-based outcome evaluation is not possible if the outcome measures are not tested. Looking at the veterinarians' combined mobility index in studies III-V, we can already identify some potential weak points. Of the combined score, 66% was related to jumping and traversing stairs, activities that dogs clinically may undertake with either excessive or minimal vigor in stressful situations. Some dogs will not jump or attempt stair-walking at the hospital, while other dogs jump, although they would never do so at home. Thus, a scale based on these activities might not be valid and/or reliable. We need a good, easy to use, valid and reliable gold standard veterinary used outcome measure for dogs with chronic pain. Then we could use this scale also to compare other scales with, but at the moment there is no such.

### **6.2.3 Plasma hormone assays**

Our intention was to monitor plasma hormone concentrations in a realistic clinical setting, and therefore, we used the forelimb vein, the *vena saphena*. In many studies, blood samples are obtained via a pre-placed jugular catheter to minimize the effects of stress on the dog. Baseline hormone concentrations of the control dogs' were compared with baseline concentrations in healthy dogs obtained from other studies (Hauptman et al. 2000, Väisänen et al. 2002) and were found to be similar, albeit not identical. Although there were significant differences in hormone concentrations between the groups with and without chronic pain, large individual variations made it impossible to define concentrations that would specifically indicate chronic pain. The stress of transporting dogs to the hospital may have had some influence on the results in our study,

as for example dogs that are new arrivals at an animal shelter are reported to have higher cortisol concentrations than those resident for a longer time (Hennessy et al. 1997). Thus, inclusion of physiologic measurements, such as plasma hormone concentrations, does not seem to provide much additional information regarding chronic pain in individual dogs.

#### **6.2.4 Radiographic examination**

Since exostosis and bone chips in any region of the coxofemoral joint were the only radiological findings associated with any of the measured chronic pain outcome variables, ie. with owner evaluated locomotion VAS, these are possibly associated with a physical restriction of movement because of the bony, advanced OA lesions in that joint. Since the appearance of the acetabular fossa correlated with most other variables in our study, it can be postulated that this may be one of the last radiographically detectable changes in the development of CHD.

Our radiographs were taken with the legs fully extended in the traditional official hip position but in evaluating laxity of the hip joint, the Penn-Hip distraction index has proven to be more accurate (Smith 1997). However, none of these methods has been shown to detect dogs with clinical pain symptoms (Smith 1997). In no studies known to us, radiological findings of coxofemoral OA has, in fact, been correlated to clinical symptoms or chronic pain, it is just accepted as a clinical truth that they do not correlate. What we also found surprising was, that no correlation existed between the duration of the clinical signs of CHD and the severity of any of the 13 evaluated radiographic changes. However, this lack of correlation is also accepted as a clinical truth. Hence, the inclusion of radiographic data did not seem to provide much additional information regarding chronic pain in individual dogs.

#### **6.2.5 Force plate analysis**

The force plate was not evaluated as a pain-assessing variable as it is generally perceived as the golden standard (Quinn et al. 2007), but it was used as a treatment outcome variable in the second clinical trial (IV,V). The limb measured to have the least PVF at  $W_0$  according to the force plate, was used in statistical analyses. The change from baseline to end of treatment in PVF and vertical impulse was in our studies within the same range as previously documented (see **Table 9**, given as medians); Budsberg et al (1999) recorded the change in PVF and vertical impulse from baseline to end of treatment as 1.6-2.3% and 0.13-0.22% of the dogs' body weight, respectively, after an 8-day analgesic treatment in dogs with OA of the hips. However, as our ranges for the medians (see **Table 9**) were quite variable and large, we did not find the method to be as valid and reliable as other researchers have done. Realizing how minor the changes are that indicate outcome, we can see that even small confounding factors may influence the results.

The following variables have been shown to introduce variability to force plate data: the dogs' body weight as well as velocity and acceleration of the subject (Riggs et al. 1993, Roush & McLaughlin 1994), gait used (Voss et al. 2007), trial repetition (Budsberg et al. 1993, Jevens et al. 1996), interday testing (Rumph et al. 1999), limb symmetry (Budsberg et al. 1993), handler (Jevens et al. 1993), breed (Poy et al. 2000), selection and habituation of the subject (Rumph et al. 1997) and individual morphometrics (Budsberg et al. 1987, Jevens et al. 1993). We tried to standardize velocity and acceleration, but used the owners as handlers, which amplified the variability. Another source for variability in our trial was due to size of the dog as we had many different breeds, body conformations, and body weights represented. We had to see that individual dogs kept the same speed thru the trial but the speeds varied some between dogs. This might have had an impact on group differences. Also, some dogs had to run tens of times before obtaining sufficient acceptable runs for both sides, giving very different values at the beginning and the end, when tired. Others have had similar problems, as it in a similar study was stated that each dog had to trot over the force plate a maximum of 75 times/session (Trumble et al. 2004). The non slippery rubber mat in our setting was probably not increasing variability as different surface (linoleum or carpet) has been investigated and did not have an impact on results (Kapatkin et al. 2007b). Our computer program initially accepted only whole kg body weight and as the value is a percentage of body weight and the change is marginal, this probably had some impact on our results as it led to at least a small change in body weight of the dogs between baseline and following evaluations. Our patient material was very heterogeneous, with dogs suffering from either CHD or ED, uni- or bilateral disease, minor or severe lameness. Some were so lame that no recordings were possible from the force plate. Thus, even when standardizing most of the potential confounding variables, the SDs were marked.

In conclusion, after this, for us first study using a force plate, which yielded results indicating problems with confounding factors, our confidence in this method as the golden standard outcome measure is limited (IV,V). As it is difficult to standardize all influencing factors in clinical research trials, especially when using heterogeneous owner-owned different breed dogs, we feel that force plate evaluation should maybe not be considered the gold standard. Also Brown et al. (2007) pointed out that it relies on relatively strict inclusion criteria, which is a problem in clinical settings. However, with a more homogeneous group, more experience, more staff i.e. a handler that runs with all dogs, and more time per patient, this method may serve us better in the future.

## 6.3 Discussion on clinical research of OA

The three tested treatments will be discussed here in light of treatment outcome and possible mechanisms of action.

### 6.3.1 Common problems in clinical research of OA

Despite the small number of dogs per group and the large variation between individual animals, both the GLM and the HCP improved symptoms of OA significantly better than the placebo. However, the variation between individual dogs within groups was marked and thus increased the confidence intervals and subsequently may have reduced the significance between treatments. The factors due to force plate analyses were already discussed but many other factors may also have increased the variation.

Firstly, it makes a big difference whether we are dealing with unilateral or bilateral disease and in our studies we allowed both. Dogs compensate differently depending on what leg/which legs are painful to use. In reality, it might even happen that when choosing the worst affected limb at the start of the trial, one might have chosen the “wrong” leg, as pain status does not correlate with radiographs, as 30-40% of dogs with OA suffer from multiple joint involvement (Olsewski et al. 1983) and as damage to one hind limb may lead to secondary lameness in an other leg as force is redistributed to the contralateral limb when the quadrupeds muscles modify their compensatory loading over time (Budsberg 2001). If we have multiple legs involved, the compensation might tire other legs, showing up as lameness or subnormal force plate results for some other leg. All of these shifts can be seen as altered results and hence, even the stage of disease when a dog is taken into the study, is relevant.

Secondly, OA is undulating, being better and worse at different times. In most articles covering the issue, weather is known to have an impact on OA; cold, damp and low barometric pressure will worsen OA pain symptoms (Guedj & Weinberger 1990, Aikman 1997, Strusberg et al. 2002, Verges et al. 2004, Liu et al. 2006), although there has also been some research that has not been able to show any correlation between weather and pain (Sibley 1985). Reversely, patients suffering from OA usually improve as the weather becomes warmer and dryer. As a consequence, one should be able to see these typical weather patterns in the dogs in the placebo groups. In the latter trial (IV,V) one could in fact see this as a trend in the placebo group dogs as they worsened or stayed the same during the cold winter months and got relief when the weather became warmer and dryer in spring. The owners in the first trial (III) filled out the first four weekly questionnaires during a period of constantly poor (subzero temperatures and unstable) weather in March/April, but according to the owners in both groups, their dogs' greatest improvement in locomotion was at this time, during the first two weeks posttreatment, despite cold and damp weather.

Thus, the improvement seen at this time in both groups suggests that it could have been caused by the treatment.

Thirdly, measuring actual clinical response to treatments in studies for OA is difficult. The subjective and semi-objective assessments in studies can mask a large amount of errors, as patients do not behave in the same way at all times. When evaluated at a hospital by researchers, the dogs are in a strange environment, where they tend to be apprehensive, nervous, or excited, therefore masking signs of pain (Dobromylskyj et al. 2000). Memories from a previous visit can make dogs either happy or uncooperative, either exaggerating or masking the variables assessed (e.g. pain, willingness to jump, allowing hind limb extension) (Dobromylskyj et al. 2000).

Fourthly, even if a treatment is successful, there may still be loss of extension and abduction and the gait may be affected due to bony changes and changes of the cartilage (Edge-Hughes & Nicholson 2007). There may also be a certain degree of habit involved, e.g. an acquired abnormal gait will not disappear immediately even when the cause of the pain is removed (Edge-Hughes & Nicholson 2007).

Fifthly, repeated visits to the hospital may reinforce general advice such as that on nutritional requirements, weight control, exercise modification etc. (Carmichael 2005). This may benefit dogs unequally; thus, emphasis should be placed on giving all owners the same information.

Last we have a problem that influence all research; placebo effect. In clinical trials of a chronic debilitating disease, a positive outcome is obviously desired. The desire for improvement tends to make evaluators answer more positively, and a positive placebo effect may be seen, especially in owners' questionnaire responses. To counteract this bias and all other bias from factors that might affect dogs in the study, randomization and a negative control group that received the treatment as a sham treatment or the product as a placebo product, were used in all of our clinical trials (III-V). Although variation should not influence results between groups in a randomized placebo controlled clinical trial, it might still influence results despite groups.

Positive controls are used when two reference points for clinical outcome evaluation are desired, and when testing for bioequivalence. When doing research on complementary treatments and remedies it is even more important to use two control groups, as positive results in these disciplines are not readily accepted. Both, showing that a treatment is better than a placebo and evaluating its clinical relevance, are warranted. In a chronic pain trial like ours, an established canine analgesic should be used as a reference (IV,V) (Holtsinger et al. 1992, Vasseur et al. 1995). The Helsinki Convention (WMA 1964) states that human placebo groups not should be used if one can use equivalence groups and these ethical principles are often referred to, also concerning animal studies. However, we still feel

that it is very important to use both placebo and positive control groups when we are dealing with treatments where positive results are not readily accepted by the profession. If they are left out, it will be near to impossible to do good, objective research on controversial treatments such as homeopathy. Therefore we recommend the use of placebo groups in both human and animal CAM research, providing that there is no risk of serious or irreversible harm. In a newer clarification of the Helsinki convention, paragraph 29, this is nowadays also stated (WMA 2002).

### **6.3.2 Gold bead implants (III)**

In the first clinical trial (III) the hypothesis was that implanting small pieces of gold wire near the hip joint would alleviate signs of pain due to OA in dogs. However, a similar result was yielded for both the treatment and the control group; there was an improvement of some 53 to 65% in both groups. The percentage in the treatment group was slightly lower than expected compared to both previous and new studies. As the veterinary evaluation in our study was quite strict, (to be considered “improved”, none of the five parameters could be negative), this could possibly have led to a lower rate of improved. Recently two similar studies including placebo groups have been reported, one indicating success of the technique (Jaeger et al. 2006, 2007), the other failure (Bollinger et al. 2002).

Jaeger et al. (2006) found 83% of gold bead-implanted dogs to have a 65% reduction of pain, whereas 60% of placebo dogs had a reduction of only 36% six months after the treatment. Comparing these results with ours, 60% of their control dogs also got “treated”. Jaeger et al. also used a placebo group where five big needles penetrated the skin at nonacupoints close to the hip. Bearing in mind the recent f-MRI studies where all kinds of needle penetrations, regardless of point location, led to analgesia (Cho et al. 2002a,b), it seems possible that both Jaeger et al.’s and our study suffered from the same type of placebo group flaw, where the placebo group unintentionally became a second treatment group. Acupuncture has long had a problem concerning placebo interventions. Researchers have therefore now suggested new types of sham acupuncture treatments, for example a needle that retracts into the shaft and does not penetrate the skin (Park et al. 2002, McManus et al. 2007). A “profile of quality of life in the chronically ill”-index gave significantly better results for the real acupuncture group than for the group that used this sham-device in conjunction with NSAID treatment, for humans with knee OA (Vas et al. 2004). Other type of study designs, such as randomized pragmatic study designs or randomized cluster designs, have also been suggested (Paterson & Dieppe 2005).

The size of the needle is also important. In both Jaeger et al.’s and our studies, the needle used for both groups was 14 G ( $\varnothing$  1.2 mm). A normal acupuncture needle ( $\varnothing$  0.25-0.38 mm) is supposed to give a subtle

treatment, whereas very thick needles, according to ancient Chinese texts are used only to get drastic results, as for paralyzed patients, etc. (Veith 1973). As pain inhibition outside the painful area varies directly with the stimulus intensity (Helms 1995), the big needles possibly induced analgesia easier than had they been very thin. However, the same placebo effect has frequently been reported also in human studies, even with thin needles. Gaw et al. (1975) found that sham-acupuncture (by normally needling, at nonacupuncture placebo points) and real acupuncture both gave significant pain relief ( $P < 0.05$ ). Thomas et al. (1991) reported that sham-acupuncture (only very shallow intra-dermal needling) gave nearly as good pain relief ( $P < 0.05$ ) as real acupuncture ( $P < 0.005$ ), compared with diazepam ( $P < 0.05$ ) and placebo-diazepam (no significant pain relief).

As we used only one piece of gold per point and only implanted three points, this might have lead to a smaller effect than other researchers have reported. Jaeger et al. (2006) used two gold wire pieces per site and implanted five points, thus, a total of 10 beads per hip. Danscher (2002) showed that the gold wire pieces at least partly induced their effect by losing gold ions that have an influence on pain via anti-inflammatory mechanisms. He also showed that the more gold implanted, the more ions could be found over a bigger area, possibly alleviating OA more. In a continuing study, Jaeger et al. (2007) found that the pain-relieving effect of the gold implants continued throughout a two-year follow-up period. Our follow-up study had similar results (Hielm-Björkman 2003).

Another controlled double-blinded study on gold implants for OA showed that the acupuncture group, according to force plate measurements, actually was statistically worse after a one-month follow-up, but there was no significant difference between groups (Bollinger et al. 2002). The lack of treatment success in their study was similar for all assessed treatment outcome variables; only 10-30% of dogs improved in treatment and control groups. These results contradict all other studies and anecdotal results to date. The small group size in Bollinger's study may have weakened their results; in the present study we had 19/19, Jaeger et al. 36/42, and Bollinger et al. only 9/9 dogs in the treated/placebo groups, respectively.

In some earlier reports, the treatment seemed to work best in young dogs with minimal bony changes (Durkes 1994, Klitsgaard 1995). In our study, 40% of the dogs had a history of pain for more than two years. Many dogs (22%) had also previously been operated on without success. The age distribution of our patients was also very different from that of Klitsgaard (1995); we had 40% of dogs aged under four years in our gold implant study, while Klitsgaard had 70% of their dogs aged under four years. The age distribution was not reported in Jaeger et al. (2006) or in Bollinger et al. (2002).

### 6.3.3 GLM

Our results of the GLM study are in agreement with those of the newer human and animal trials. In the early GLM clinical trials on human patients suffering from OA, the outcomes were not very good and often contradictory (Gibson et al. 1980, Huskisson et al. 1981). However, 20 years later, possibly after having stabilized the product by freeze-drying and lyophilizing (Broadbent & Kosuge 1985), the results of clinical trials for GLM have been much more promising (Gibson & Gibson 1998, Bierer & Bui 2002, Cho et al. 2003, Pollard et al. 2006, Lawson et al. 2007). The product tested here is a version of this newer stabilized powder product.

Four studies have been published on stabilized GLM in the treatment of canine OA, and our findings are consistent with three of them. Bierer and Bui (2002) conducted three six-week, randomized, double-blind trials in which they compared three different GLM dog feeds with control feeds. As in our study, all individual variables in Bierer and Bui's study did not show a significant improvement, although a significant change was seen in the total arthritis score in favor of the three GLM test groups. Of the eight variables evaluated, only two (joint pain and joint swelling) were significantly improved at week 6 in the GLM groups in all three trials, and joint crepitus improved significantly in two of the trials. GLM thus seems to have an effect first on the joint surface, whereas secondary positive effects such as mobility and ROM, are seen later.

A multicenter field study - with no placebo groups - testing a GLM supplemented dry diet, showed statistically significant positive outcome results, that were similar to ours (Servet et al. 2006). At the end of the 50 day trial, veterinarians evaluated 94% of dogs to have improved arthritic signs and evaluated 3 different negative scores to be reduced by 33-36% from baseline (Servet et al. 2006).

A third double-blind, placebo-controlled canine trial studied a slightly different product that ours, but also had results that complied with ours. They used a product containing GLM and brewer's yeast, with the placebo made of brewer's yeast and dried fin-fish (Pollard et al. 2006). However, the use of this placebo group renders the trial results unreliable in our opinion, as one would expect the placebo group to improve at least to some extent, as cartilage, bone, minerals and fatty acids from the dried fish (Anthony et al. 1983) all might stimulate cartilage and bone metabolism and as brewer's yeast can stimulate both mobility and mental activity (Hielm-Björkman et al. 2007). This effect was also seen in the results, as the pain scores decreased in both groups. However, even with their poor study design, the improvement continued throughout the study period (112 days), showing a significant positive result for the GLM group from day 54. The slow onset of effect was also seen in our trial, where the effect continued to grow slowly through the whole study period, even after cessation of treatment, and could be seen e.g. as minimal use of extra carprofen at the

W<sub>12</sub> follow-up. GLM has been shown to have a slower onset (Audeval & Bouchacourt 1986, Cleland et al. 1988, Kremer 1991, Volker & Garg 1996) and a longer effect (Gibson & Gibson 1980) than fast working NSAIDs. One of the first human studies indicated that the beneficial effects of GLM treatment could last for 2-3 weeks after cessation of therapy, if given at least for two months (Gibson & Gibson 1980).

From all of the GLM results, we can conclude that the study period should be long enough to be able to detect secondary changes, like improvement in mobility. Human studies that tested similar fatty acid products were unable to show a significant improvement compared with controls before patients had ingested test products for at least 3-6 months, indicating the slow onset of the effect (Cleland et al. 1988, Kremer 1991, Volker & Garg 1996, Cobb & Ernst 2005).

Apart from the fatty acids, the other main ingredient in GLM is chondritin sulphate (that is made up from glucosamine and galactosamine) so results from studies on these ingredients also interest us. A double-blind, controlled study testing a glucosamine/chondroitin sulphate combination for dogs suffering from OA also had a result that was in agreement with ours; it showed a significantly positive treatment response, but only at day 70 (McCarthy 2007). Another glucosamine/chondroitin sulphate trial for OA dogs showed no effect, but here the treatment period was only 60 days (Moreau et al. 2003). This indicates that also treatments targeting the cartilage matrix need a long time to show effect.

In the fourth GLM trial for dogs suffering from OA Dobenecker et al. (2002) used a smaller dose of GLM, only 25% of the dose used by Bierer and Bui (2002), Servet et al. (2006) and our initial dose. No improvement was found in dogs fed GLM compared with the placebo group, possibly indicating that our dose, as well as the doses of the other above presented studies, were more effective than that of Dobenecker's group.

#### **6.3.4 HCP**

Our results showed that four of the six measured variables showed a significant difference in the HCP group compared with the placebo group in number of improved subjects per group or in improvement rate overall, and mostly in both. This was somewhat surprising but interesting.

There is a growing body of research that supports homeopathic medicine, regarding both basic research on mechanisms of action and clinical trials (Bellavite 2006b). On a more general level, our results are in accordance with Linde et al. (1997), who in the *Lancet* had reviewed 89 randomized, placebo-controlled trials in a thorough meta-analysis. A statistically significant difference between the groups in favor of homeopathy was evident. The groups receiving homeopathic treatments had a combined odds ratio of 2.45 (95% CI 2.05, 2.93), the odds ratio for the 26 best-quality studies being 1.66 (1.33, 2.08), and, after correction for publication bias,

1.78 (1.03, 3.10). Of the trials included, 67% reported a positive outcome for their trial group. Two other meta-analyses published in the British Medical Journal and Lancet yielded similar results (Kleijnen et al. 1991, Shang et al. 2005). Other meta-analyses have found no positive effect of homeopathy (Ernst 2002, Altunc et al. 2007). A “bias of the observer” has been shown to exist when choosing trials for these meta-analyses, possibly partly explaining these different results (Frass et al. 2005, Kiene et al. 2006). However, in many meta-analyses it has been shown that more rigorous trials tend to yield less optimistic results than trials with less precaution against bias (Linde et al. 1999, Shang et al. 2005), but there is no linear relationship between quality scores and study outcome (Linde et al. 1999).

Our results compare well with a trial published earlier this autumn where Zeel® *ad us. vet.* was compared to carprofen treatment in dogs with OA (Neumann et al. 2007). In this study Zeel® was statistically equivalent to the positive control carprofen at all evaluation times (days 28, 56 and 70) and the effect persisted longer in the Zeel® group than in the carprofen group after discontinuation of the treatment. A big difference between the Neumann et al. study and ours was dose and mode of treating. We used a lower dosing than that used in the Neumann study, which is the one currently recommended. The recommendation at the beginning of our study was giving only ½-1 ampoule once per day, which is 1/3 of the now recommended dose. We also used a drinkable ampoule, whereas the Zeel® *ad us. vet.* nowadays comes as a pill. Whether these modifications had an impact on the results is unknown but in conventional pharmacology one can suppose that a dose is increased if it has been shown to be too low but this might not be the case for homeopathical therapies. We have found no publication on this.

Our results are also comparable with some double-blinded research that has been done with HCP Zeel® in human and equine patients suffering from OA. A therapeutic equivalence was found between the NSAID Diclofenac and Zeel®; after six weeks, the treatment outcome of 47% of the human group receiving Zeel® and 51% of the group receiving Diclofenac was evaluated as good/very good (Maronna et al. 2000). In two other comparative trials Zeel® comp. was found to be as good as hyaluronic acid for human OA (Nahler et al. 1996) and Zeel® *ad us. vet.* was found to be equivalent to hyaluronic acid for equine OA (Faulstich et al. 2006). Our results compare with a human trial with knee OA patients (n=592) where Zeel Comp. N at the six week check-up was found to be noninferior to treatment with two coxibs at an equivalence limit of 10% (one-sided probability of error = 0.025)(Birnesser et al. 2003). However, none of these trials included placebo groups, referring to the equivalence study guidelines (WMA 1964).

No adverse reactions were seen in our study, as is usually the case

with homeopathic remedies. However, in low-dilution remedies, some ingredient might cause a reaction if the patient is allergic to it, e.g. a reaction towards Zeel® may arise because of *rhus toxicodendron* (Poison Ivy), a poisonous plant.

Another aspect of this particular study still has to be addressed. The fact that the product tested is a low-dilution homeopathic remedy and actually includes measurable amounts of ingredients does for many mean that the product is not a “true” homeopathic remedy. As described in the literature review; all products that treat a disease symptom according to the *similia* principle and are diluted from a homeopathic mother tincture using the potentizing method, are homeopathic remedies. All are not voided from material other than water or sugar, however. The fact that Zeel® *ad us vet.* actually includes materia in measurable amounts could in fact explain our good results in a way that would satisfy both the homeopath and the sceptic. It might be possible that the summed amount of very small amounts of ingredients with anti-inflammatory properties could indeed result in a detectable anti-inflammatory effect. Birnesser & Stolt (2007) recently published a nice review looking at research on the individual constituents of Zeel®. *Arnica Montana*'s constituents helenaline and dihydrohelenaline have been shown to possess anti-inflammatory properties (Mascolo et al. 1987) and *Solanum dulcamara* was shown to highly inhibit platelet activating factor-exocytosis (Tuno'n et al. 1995). This aspect is also supported by the research where high-dilutions, 12c, 30c and 200c of *Rhus Toxicodendron* showed smaller and non-significant effects on induced rat and mice edema whereas a significant anti-inflammatory effect was present with a 6c dose (dos Santos et al. 2007). The amount of *Rhus Toxicodendron* substance in this 0.5 ml oral dose of 6c dilution was, however, very small. Valentiner et al. (2003) had also found that the inhibitory effect of cell proliferation of *Rhus Toxicodendron* at D1-D2 was significant, but the more diluted doses were not significantly effective anymore. In a meta-analysis Lüdtko and Hacke (2005) looked at homeopathical *Arnica Montana* studies and found a significant OR on effectiveness for the evaluated trials but also here, the better quality studies were less likely to report positive results. We can here only conclude that it would be interesting to know if the amount of materia in our tested product actually was the reason to our treatment success or if it would have worked also in higher dilutions, as a “classic” homeopathic remedy.

### **6.3.5 Statistical methods**

In the gold implant study (III), unpaired two-tailed parametric tests were used, according to the original study protocol. When setting up the second study, we planned to compare the groups with respect to their change in mean between  $W_0$ - $W_8$  for each treatment outcome variable. All of the data could not, however, be analyzed according to the original study protocol

so the data in the second trial (IV,V) was converted into dichotomous variables. This can be criticized, as it marginally may increase the chances of getting a more positive outcome in a study but we justify this conversion by having four of the 15 dogs in the placebo group using rescue analgesia, i.e. extra NSAIDs 3-5 times per week or more, at the end of treatment, at our most important point of treatment evaluation,  $W_8$ . This probably led the placebo group evaluators to answer the questionnaire differently than if the dogs had not taken the extra carprofen. Also, lameness and force plate measurements were likely to be inaccurate, as nearly 30% of the group probably showed less lameness and used more ground reaction forces than if they had not taken extra analgesic medication. In fact, they were probably showing treatment effects that were closer to those of the carprofen group than to the no-treatment placebo group. The placebo group, thus, both did and did not receive effective analgesia, and we could neither use these results while calculating means, nor did we want to exclude them. That all four dogs taking extra analgesia came from the placebo group is already a result to be considered, and the only way all data could be included, was to convert the results into dichotomous responses. As this result did not express the degree of clinical improvement, this was tested for separately and again we wanted to use the whole data. The change of values for the dogs using the rescue analgesia was justified with the assumption that although some other owner's dog also was in similar pain, this other owner had decided that their dog would be ok without the rescue carprofen. As we gave the same value to four of the placebo dogs and thereby not had a normal distribution at  $W_8$ , we had to use a nonparametric test when evaluating range of improvement. Although significance is harder to show with nonparametric tests (Greenhalgh 1997), we had significant differences with both GLM and HCP treatments compared with placebo.

### **6.3.6 Working mechanisms of treatments used in this study**

Basic research provides possible mechanisms of action for all three of the treatments used, therefore indicating that our positive treatment results may be true. As the most attractive new strategies for really "treating" OA have been to target (1) the inflammatory process (by cytokines such as IL-1 $\beta$ , NO, reactive oxygen species and eicosanoids), (2) the cartilage degradation (by MMP-13 and Aggrecanase-2), (3) the subcondral bone remodelling (by biophosphonates) (Pelletier et al. 2006) and (4) by controlling gene expression (Gelse et al. 2005), we seem to have several of these as mechanisms of action in our tested treatments.

GLM and acupuncture significantly reduce pro-inflammatory cytokines IL-1, IL-6, IL-12p40, TNF- $\alpha$ , and anti-collagen IgM antibodies, IgG levels (Mani & Lawson 2006, Yim et al. 2007, Lawson et al. 2007). IFN- $\gamma$  can decrease after acupuncture stimulation (Yim et al. 2007). Acupuncture has been shown to increase T-, B-, and NK-cell activity (Yamaguchi et al.

2007). GLM may also mediate T-helper cell regulation (Mani & Lawson 2006), and has been shown to ameliorate CIA in mice by decreasing INF- $\alpha$  and other catabolic pro-inflammatory cytokines (Lawson et al. 2007). Ingredients in the HCP product may inhibit fibroblasts and leukocyte elastase activity (Stancikova 1999b, Valentiner et al. 2003). Research also suggests anti-inflammatory and immuno-regulatory effects similar to those in dual inhibitor NSAIDs; both GLM and the HCP have been established to inhibit prostaglandin synthesis by COX-1 and COX-2 enzymes and leukotriene production by 5-LOX (Whitehouse et al. 1997, Jäggi et al. 2004, Mani & Lawson 2006).

All the above mentioned mechanisms plus many more reviewed in the literature review before, would indicate that the researched treatments indeed could be beneficial in the treatment of OA.



## 7. CONCLUSIONS

Trials aimed at finding and testing suitable treatment outcome measures and measures of chronic pain to be used for assessment of chronic pain in clinical trials of treatments for canine OA led to the following conclusions:

1. There are many different ways to evaluate canine chronic pain in research, but only a few of them have proven to be both valid and reliable. Examination using radiographs, plasma stress hormones, or force plates could not with certainty identify dogs suffering from chronic OA pain. The pain and locomotion VASs should be evaluated further. The MDS questionnaire proved to be the most appropriate of the methods tested. The 11 most relevant questions, which were also easy to answer for all kinds of owners, with all types of dogs and living in all types of home environments, were combined to form the Helsinki chronic pain index (HCPI).
2. The Helsinki chronic pain index – HCPI - was evaluated and found to be a simple, reliable and valid canine pain questionnaire. It was validated against sound dogs and was repeatable under identical conditions (reliability), sensitive to changes in pain (responsiveness), and, according to the principal component analysis, had a stable single component structure. We propose that the index be used as an outcome measure in both research and clinical work to evaluate chronic OA pain, by owners.

Trials testing three different treatments for canine OA using randomized, controlled double-blind study design led to the following conclusions:

3. In the first trial, gold wire pieces implanted around the coxofemoral joint in dogs with OA due to CHD did not show a significant difference in reducing pain between the treatment and sham group. As 53-65% of both groups improved, the placebo group may have not been a real “no treatment” group, instead possibly behaving like an acupuncture group. Also, big variability or changes in the disease pattern of OA may have influenced the results. More research is indicated before any recommendations can be given.

The second trial tested a green lipped mussel product and a low-dose homeopathic combination preparation in the treatment of OA pain.

Both products improved significantly more dogs in the treatment groups than in the placebo groups, and overall improvement was significantly better in both treatment groups compared with the placebo group, although they were not as effective as our positive control, carprofen. As no side-effects were seen, both green lipped mussel and the combination preparation Zeel® *ad us vet.* may be beneficial for use in treating canine OA, especially for dogs that either cannot tolerate NSAIDs due to their side-effects or that require long-term management.

## 8. EPILOGUE

When conducting research on complementary treatments, rigorous trial design is of the utmost importance. Many New Age healers or complementary medicine practitioners - while roundly denouncing medicine - seek medicine's cover to make their own miraculous claims respectable. As the domain of medicine is defined by certain goals, principles, measure of outcomes, and standards of evidence (Schneiderman 2000), "complementary" medicine should be defined similarly, to get the same status. This new positive attitude toward research can already be seen in CAM studies of today; study quality is rising rapidly.

When using animals as patients, the placebo response to the treatment is considered to be lower than when using humans. Animals have been used in pre-clinical drug testing for decades and are especially valuable in research of diseases that have a similar pathology in animals and in humans. The dog, suffering from a very similar OA to man, is an excellent species to use while testing new treatments for this disease. Dogs have been used extensively in this research, particularly as the Pond-Nuki model of OA (Pond & Nuki 1973). At the same time, we as veterinarians can benefit from their trial results and use the obtained knowledge for the benefit of our patients.

Our results may indicate that sham-acupuncture and gold bead implants, as well as carprofen, GLM, and HCP, have significantly positive effects on pain and lameness, due to canine OA. But, as evidence-based medicine requires a multitude of well-conducted high quality trials to be able to evaluate each of these treatments in large meta-analyses, and as our findings come from small studies and are among the first published RCTs on these treatments for this condition, we are, at this time, unable to give any evidence based recommendations about their benefits. But, as the fruits from this labor will not be at our disposal for another decade or so, at least there are now more and more of us that work in this controversial field. With time, a RCT derived critical mass point will tip these therapies over; some this way, some that way.



## ACKNOWLEDGMENTS

The studies included in this thesis were carried out at the Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, Helsinki University Small Animal Hospital. Laboratory analyses were also carried out at the University of Oulu.

My sincere gratitude goes to my supervisor, the head of the department of Clinical Equine and Small Animal Sciences, Professor Riitta-Mari Tulamo, and Senior Lecturer Marja Raekallio for their supportive interest in this thesis and their courage to let me do a thesis with “homeopathy” in the title. I especially want to thank you Ritsku, for your friendship, your commitment and your invaluable help in editing my texts and you Marja, for your insightful comments and for making me take pride in my work.

I thank the pre-examiners of this thesis, Professor John Innes, University of Liverpool, and Professor Steven Budsberg, University on Georgia, as well as my extra pre-examiner, Professor Lars-Erik Appelgren, for their insights and valuable constructive criticism of this manuscript.

Many thanks to all of my co-workers during the different trials: Marja Raekallio, Erja Kuusela and Hanna Salonen as evaluating veterinarians, Johanna Keijonen, Titta Kokkonen, Anne Markkola, Marianne Saari, Erja Saarto, Tiina Torkko as students (nowadays all DVMs).

A specially big thanks goes to Hannu Rita, my highly estimated co-writer and statistician, who spent sooooo many hours trying to make me understand psychometric theory. Also, my most sincere thanks I owe to my second statistician Arto Ketola. Although I always thought I hated statistics, my meetings with you both have actually been one of the nicest parts of this thesis process. Not only has your excellent company given me pleasure, but you have also awakened the small statistician in me ;c)

I am also grateful to Professor Marjatta Snellman, Tarja Salonen and Taina Mäki in the x-ray department for their help in the clinical trials and their smiles and supportive comments throughout the project. The “girls” in the “kassa”, Satu Tanner, Satu Eskelinen, Anne Levonmäki and Riitta Luoma, I thank for help with distributing questionnaires, for making odd time reservations, and for your never ending support and friendliness. I am also utterly grateful to Matti Järvinen, Harri Kainulainen, Timo Haapanen, and Mikko Valkonen for their always friendly help with small matters that are bigger than life when they happen. I am grateful to Satu Sankari for her patient explanations and instructions on how to use the most complicated biochemical assessment machines, to Misse Väisänen for our nightly tea-sessions to discuss PhD problems, and to Faik Atroshi for showing a

genuine interest in my ideas. Also, I want to thank all of my other colleagues at the University animal hospital, all assistant staff including the cleaners who smilingly have tried to clean my chaos of a room.

A special thanks go to all the dogs and their owners for having had trust in us and for arriving so eagerly every time for the evaluations.

I warmly acknowledge Jukka Kuussaari and Allen Schoen, my mentors and role models. I thank both of you for the confidence that you had in me on this journey, as so many others considered it impossible to work in an area where mysticism and evidence-based medicine overlap.

I thank all of my friends and colleagues around the world who have commented on manuscripts, posters, or abstracts and helped me with specific problems; Phil Rogers, Stelio Luna, Gry Jaeger, Astri Hagenlund, Marcia Szabo, Erhard Schulze, Dietrich von Schweinitz, Linda Boggie, Luc Janssens, Uve Petermann, Heidi Bruncrona, Cathy and Tom McGowen, and many many more.

Finally, I owe my deepest gratitude to my family. To my parents, Börje and Kiki Hielm, who were with me along the way, but were unable to see the end-product. To my husband Ingmar Björkman, who now even defends my strange projects and results. A special thanks to you for being my best friend, for acting as a wall to bounce my ideas off of, for reading two of my papers (several times), and for being mother, father, cook, and cleaner for the family for the last 6 months. I will try to finish my next thesis with less interference on home chores. I love you. To my three lovely children Linus, Troy and Egil, who have kept me focused and happy along this journey, I owe more than I can say. A special thank you goes to my sisters and brother, with families; Maria Mäkinen, Lotta Antman, and Sophia Jansson, who have supported me all the way and my brother, Sebastian Hielm, who has trained me for my defense on numerous occasions. I love you all.

Another special thank you goes to all the people to whom I've not given the time they deserve because of "some stupid work" that was suddenly given priority, especially Nina Linder, Daniela Wrede, Jasu Saksela, Camilla Moberg, Miku Ahlström, Marcus Brunkrona, Beni Airas, Priggan Prigorowsky, Bitte Slotte, Jean-Luc Laxe 'n, Ritva Krokfors, all with families, as well as Pelle and Maritta Björkman, Thua and Kurre Moberg and Ruttan Dahlberg.

I am indebted to Vetcare, the Finnish Kennel Club, Pfizer, Boeringer Ingelheim, Heel, and Biofarm, for the means to purchase a force plate for the University Hospital and for medicines and placebos received for the trials. Financial support received from the Helvi Knuutila Foundation and Finnish Foundation of Veterinary Research is greatly acknowledged.

# APPENDIX

## Appendix 1. Psychometric properties of a scale

For a scale to be of use, it has to be both valid and reliable and this is evaluated by its psychometric properties (Cronbach 1951, Siegel 1956, Nunnally 1978, Carmines & Zeller 1979, Cohen 1988, Kline 1993, Streiner & Norman 1995, Johnston 1998, DeVellis 2003, Tabachnick & Fidell 2007).

**VALIDITY** is the quality of a scale, its ability to measure what it is supposed to measure. Validity can be divided into four different types: face validity and content validity rely on the internal logic of the measure; criterion validity and construct validity are less subjective and more empirical.

**Face validity** is the extent to which the scale or index is subjectively viewed by knowledgeable individuals as covering the concept, e.g. that each variable in the questionnaire measures chronic pain in some way.

**Content validity** is related to face validity, being based on logic and expertise. It asks whether the scale or index covers all of the generally accepted variables of for example, chronic pain, i.e. is it sufficiently comprehensive?

**Criterion validity** is used when describing the correlation between a scale and another, already validated external measurement, of the same phenomenon.

**Construct validity** has to do with the ability of the scale or index to measure variables that are theoretically related to the variable that the scale purports to measure.

**RELIABILITY** refers to the extent to which the measure yields the same score each time it is administered, all other things being equal. There are four types of reliability: Repeatability, inter-rater reliability, internal consistency reliability, and responsiveness. Thus, reliability can be measured in different ways and is always inexact but more tests done, strengthen the index.

**Internal consistency** or **equivalence** is when the reliability of the instrument is judged by estimating how well the items that reflect the same construct yield similar results or how consistent the results are for the different items for the same construct within the measure and the Cronbach's coefficient  $\alpha$  (Cronbach 1951) is the best known method for

evaluating this. The cause for unreliability may lie in one or more questions being vague or confusingly worded. As a result, the reader's interpretation at the second reading may have differed from the initial interpretation of the same variable.

**Repeatability** (also called **stability**, **test-retest**, **temporal reliability** and **intra-observer reliability**) is when a test is given twice to the same people and thereby evaluated by the test-retest method. When the measure is taken over intervals of time, the scores of the owners should remain consistent. This is often tested using intra-class correlation (Streiner & Norman 1995) but also many other tests are possible, e.g. Cohen's Kappa (Cohen 1988), Spearman- (Siegel 1956) or Pearson- (Streiner & Norman 1995) correlation tests.

**Inter-rater reliability** (also called **Inter-observer reliability**) is when two observers rate the same phenomenon at the same time, e.g. two veterinarians evaluating the same dog at the same time using the same scale.

**Responsiveness** (also called **sensitivity to change**) of the scale reflects the capability of the instrument to measure changes in levels of pain over time, in particular, in responses to clinical interventions such as analgesics.

**Cut-off points** on pain scales are points on the scale that differentiate between mild, moderate, and severe pain, between pain being tolerable and intolerable, thus indicating where (additional) analgesics are needed because the dog is experiencing too much pain. Cut-off points must be reliable and valid for each population.

## Appendix 2:

### HELSINKI CHRONIC PAIN INDEX

Name of Dog \_\_\_\_\_ Owner \_\_\_\_\_ Diagnosis \_\_\_\_\_

Date \_\_\_\_\_ Questionnaire no. \_\_\_\_\_

Tick only one answer – the one that best describes your dog during the preceding week

						Points
1. Rate your dog's mood:	Very alert	alert	neither alert, nor indifferent	indifferent	very indifferent	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
2. Rate your dog's willingness to participate in play:	Very willingly	willingly	reluctantly	very reluctantly	does not at all	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3. Rate your dog's vocalization (audible complaining, such as whining or crying out):	Never	hardly ever	sometimes	often	very often	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
4. Rate your dog's willingness to walk:	Very willingly	willingly	reluctantly	very reluctantly	does not walk at all	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
5. Rate your dog's willingness to trot:	Very willingly	willingly	reluctantly	very reluctantly	does not trot at all	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
6. Rate your dog's willingness to gallop:	Very willingly	willingly	reluctantly	very reluctantly	does not gallop at all	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
7. Rate your dog's willingness to jump (eg. into car, onto sofa...)	Very willingly	willingly	reluctantly	very reluctantly	does not jump at all	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
8. Rate your dog's ease in lying down:	With great ease	easily	neither easily, nor difficultly	with difficulty	with great difficulty	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
9. Rate your dog's ease in rising from a lying position:	With great ease	easily	neither easily, nor difficultly	with difficulty	with great difficulty	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
10. Rate your dog's ease of movement after a long rest:	Never difficult	hardly ever difficult	sometimes difficult	often difficult	very often/always difficult	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
11. Rate your dog's ease of movement after major activity or heavy exercise:	Never difficult	hardly ever difficult	sometimes difficult	often difficult	very often/always difficult	_____
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

---

Points      0                      1                      2                      3                      4

**Total up the answers to all 11 questions. Total chronic pain index score: \_\_\_\_\_**



## REFERENCES

- ACVA - American College of Veterinary Anesthesiologists. American College of Veterinary Anesthesiologists' position paper on the treatment of pain in animals. *J Am Vet Med Assoc* 1998;213:628-630.
- Aikman H. The association between arthritis and the weather. *Int J Biometeorol* 1997; 40:192-199.
- Åkerblom S, Sjöström L. Evaluation of clinical, radiological and cytological findings compared to arthroscopic findings in shoulder joint lameness in the dog. *Vet Comp Orthop Traumatol* 2007;20:136-141.
- Allen K, DeCamp CE, Braden TD, Bahns M. Kinematic gait analysis of the trot in healthy mixed breed dogs. *V.C.O.T.* 1994;7:148-153.
- Almay BGL, Johansson F, von Knorring L, Terenius L, Wahlström A. Endorphins in chronic pain. Differences in CSF endorphin levels between organic and psychogenic pain syndromes. *Pain* 1978;5:153-162.
- Altman R, Gray R. Inflammation in osteoarthritis. *Clin Rheum Dis* 1985;11:353.
- Altman RD. Design and conduct of clinical trials in osteoarthritis. *Scand J of Rheum* 1990;suppl.81:24-27.
- Altman S. Techniques and instrumentation. In: *Veterinary Acupuncture – ancient art to modern medicine* (2<sup>nd</sup> edn.). Schoen (Ed.) Mosby Inc., Missouri, USA. 2001:95-112.
- Altunc U, Pittler MH, Ernst E. Homeopathy for childhood and adolescence ailments: Systematic review of randomized clinical trials. *Mayo Clin Proc* 2007;82:69-75.
- Anderson MA, Mann FA. Force plate analysis: A noninvasive tool for gait evaluation. *Compend Contin Educ Pract Vet* 1994;16:857-867.
- Anthony JE, Hadgis PN, Milam RS, Herzfeld GA, Taper LJ, Ritchey SJ. Yields, Proximate Composition and Mineral Content of Finfish and Shellfish. *J Food Sci* 1983;48:313.
- Aragon CL, Hofmeister EH, Budsberg SC. Systematic review of clinical trials of treatments for osteoarthritis in dogs. *J Am Vet Med Assoc* 2007;230:514-521.
- Asai T. Confidence in statistical analysis. *Br J Anaesth* 2002; 89:807-810.
- Aspden RM, Scheven BAA, Hutchison JD. Osteoarthritis is a systemic disorder involving stromal cell differentiation and lipid metabolism. *Lancet* 2001;357:1118-1120.
- Audeval B, Bouchacourt P. Etude contrôlée en double aveugle contre placebo, de l'extrait de moule *Perna canaliculus* (moule aux orles vertes) dans la gonarthrose. *Gaz Medicale* 1986 ; 93:111-116.
- Banerjee P, Biswas SJ, Belon P, Khuda-Bukhsh AR. A potentized homeopathic drug, *Arsenicum Album* 200, can ameliorate genotoxicity induced by repeated injections of arsenic trioxide in mice. *J Vet Med A* 2007;54:370-376.

- Bassleer C, Henrotin Y, Franchimont P. In-vitro evaluation of drugs proposed as chondroprotective agents. *Int J Tissue React* 1992; 14:231-241.
- Bauer JE. Evaluation of nutraceuticals, dietary supplements, and functional food ingredients for companion animals. *J Am Vet Med Assoc* 2001;218:1755-1760.
- Begg C, Cho M, Eastwood S, Horton R, Moher D, Olkin I, Pitkin R, Rennie D, Schulz KF, Simel D, Stroup DF. Improving the quality of reporting of randomized controlled trials- The consort statement. *J Am Med Assoc* 1996;276:637-639.
- Bellavite P, Conforti A, Ortolani R. Immunology and Homeopathy. 3. Experimental studies on animal models. *Evid Based Complement Alternat Med* 2006b;3:171-186.
- Bellavite P, Conforti A, Pontarollo F, Ortolani R. Immunology and Homeopathy. 1. Historical background. *Evid Based Complement Alternat Med* 2005;2:441-452.
- Bellavite P, Conforti A, Pontarollo F, Ortolani R. Immunology and Homeopathy. 2. Cells of the immune system and inflammation. *Evid Based Complement Alternat Med* 2006a; 3:13-24.
- Bellavite P, Ortolani R, Pontarollo F, Piasere V, Benato G, Conforti A. Immunology and Homeopathy. 4. Clinical studies- Part 1. *Evid Based Complement Alternat Med* 2006c;3:293-301.
- Bellavite P, Ortolani R, Pontarollo F, Piasere V, Benato G, Conforti A. Immunology and Homeopathy. 4. Clinical studies- Part 2. *Evid Based Complement Alternat Med* 2006d;3:397-409.
- Bellavite P, Ortolani R, Pontarollo F, Pitari G, Conforti A. Immunology and Homeopathy. 5. The rationale of the "Simile". *Evid Based Complement Alternat Med* 2007;4:149-163.
- Belon P, Cumps J, Ennis M, Mannaioni PF, Roberfroid M, Sainte-Laudy J, Wiegant FAC. Histamine dilutions modulate basophile activation. *Inflamm Res* 2004;53:181-188.
- Belon P, Cumps J, Ennis M, Mannaioni PF, Sainte-Laudy J, Roberfroid M, Wiegant FAC. Inhibition of human basophile degranulation by successive histamine dilutions: Results of a European multi-centre trial. *Inflamm Res* 1999;48S:S17-18.
- Bennett D & May C. Joint diseases of dogs and cats. In: *Textbook of Veterinary Internal Medicine – Volume 2* (4<sup>th</sup> edn). W B Saunders, PA, USA 1995:2053-2058.
- Bennett RL, DeCamp CE, Flo GL, Hauptman JG, Stajich M. Kinematic gait analysis in dogs with hip dysplasia. *Am J Vet Res* 1996;57:966-971.
- Bierer TL, Bui LM. Improvement of arthritic signs in dogs fed green-lipped mussel (*Perna canaliculus*). *J Nutr* 2002;132:S1634-1636.
- Birnesser H, Klein P, Weiser M. Treating osteoarthritis of the knee: A modern homeopathic medication works as well as COX 2 inhibitors. *Der Allgemeinarzt* 2003;25:261-4.
- Birnesser H, Stolt P. The Homeopathic Antiarthritic Preparation Zeel comp. N: A Review of Molecular and Clinical Data. *Explorer* 2007;3;16-22.
- Black J: Friction and wear. Forces and equilibrium. Deformation. In *Orthopaedic biomaterials in research and practice*. New York. Churchill Livingstone 1988:1-55.

- Bollinger C, DeCamp CE, Stajich M, Flo GL, Martinez SA, Bennett RL, Bebachuk. Gait analysis of dogs with hip dysplasia treated with gold bead implantation acupuncture. *Vet Comp Orthop Traumatol* 2002;2:116-122.
- Bonamin LV, Martinho KS, Nina AL, Caviglia F, Do Rio RG. Very high dilutions of dexamethasone inhibit its pharmacological effects *in vivo*. *Br Homeopath J* 2001;90:198-203.
- Borer LR, Peel JE, Seewald W, Schawalder P, Spreng DE. Effect of carprofen, etodolac, meloxicam, or butorphanol in dogs with induced acute synovitis. *Am J Vet Res* 2003;64:1429-1437.
- Bouck GR, Miller CW, Taves CL. A comparison of surgical and medical treatment of fragmented coronoid process and osteochondritis dissecans of the canine elbow. *Vet Comp Orthop Traumatol* 1995;8:177-183.
- Boyeux M. Die anwendung von Zeel® in der Behandlung der Artropatien des Renn- und Sportpferdes. *Biol Tiermed* 1984;3:50-55. (In German)
- Brandt KD, Mazzuca SA, Katz BP, Lane KA, Buckwalter KA, Yocum DE, Wolfe F, Schnitzer TJ, Moreland LW, Manzi S, et al. Effects of doxycycline on progression of osteoarthritis: results of a randomized, placebo-controlled, double-blind trial. *Arthritis Rheum* 2005;52:2015-2025.
- Brinker WO, Piermattei DL, Flo GL. Handbook of small animal orthopaedics & fracture treatment. 2<sup>nd</sup> ed. W.B.Saunders, PA, USA. 1990:355-360.
- Broadbent JM, Kosuge Y. Stabilized mussel extract. New Zealand patent 211928 (29 april 1985); Australian patent PG 4775/84 (1 May 1984) 1985.
- Brooks PM. Side effects from Seatone. *Med J Aust* 1980;2:158 (Letter)
- Brosseau L, Welch V, Wells G, de Bie R, Gam A, Harman K et al. Low level laser therapy (Classes I, II and III) for treating osteoarthritis (Cochrane Review). In: *The Cochrane Library*, Issue 4. Chichester, UK: John Wiley & Sons, Ltd. 2003a.
- Brosseau L, MacLeay L, Robinson V, Wells G, Tugwell P. Intensity of exercise for the treatment of osteoarthritis (Cochrane review). In: *The Cochrane Library*, Issue 4. Chichester, UK: John Wiley & Sons, Ltd. 2003b.
- Brown DC, Boston RC, Coyne JC, Farrar JT. Development and psychometric testing of an instrument to measure chronic pain in dogs with osteoarthritis. *Am J Vet Res* 2007b;68:631-637.
- Brown DC. Outcomes based medicine in veterinary surgery: Getting hard measures of subjective outcomes. *Vet Surg* 2007;36:289-292.
- Bucci LR. Chondroprotective agents: Glucosamine salts and chondroitin sulphates. *Townsend Lett Dr* 1994;1:52-54.
- Budsberg SC, Bergh MS, Reynolds LR, Streppa HK. Evaluation of pentosan polysulfate sodium in the postoperative recovery from cranial cruciate injury in dogs: A randomized, placebo-controlled clinical trial. *Vet Surg* 2007;36:234-244.
- Budsberg SC, Chambers JN, Van Lue SL, Foutz TL, Reece L. Prospective evaluation of ground reaction forces in dogs undergoing unilateral total hip replacement. *Am J Vet Res* 1996;57:1781-1785.

- Budsberg SC, Jevens DJ, Brown J, Foutz TL, DeCamp CE, Reece L. Evaluation of limb symmetry indices, using ground reaction forces in healthy dogs. *Am J Vet Res* 1993;54:1569-1574.
- Budsberg SC, Johnston SA, Schwarz PD, De Camp CE, Claxton R. Efficacy of etdolac for the treatment of osteoarthritis of the hip joints in dogs. *J Am Vet Med Assoc* 1999;214:206-210.
- Budsberg SC, Verstraete MC, Soutas-Little RW. Force plate analysis on the walking gait in healthy dogs. *Am J Vet Res* 1987;48:915-918.
- Budsberg SC. Long-term temporal evaluation of ground reaction forces during development of experimentally induced osteoarthritis in dogs. *Am J Vet Res* 2001;62:1207-1211.
- Budsberg SC. The randomized clinical trial. *Vet Surg* 1991;20:326-328.
- Burns DM, MacDonald SGG. In *Physics for biology and pre-medical students*. Addison-Wesley Publishers Limited. London. 1975: 419.
- Cardinet GH, Kass PH, Wallace LJ, Guffy MM. Association between pelvic muscle mass and canine hip dysplasia. *J Am Vet Med Assoc* 1997;210:1466-1473.
- Carmichael S. Putting theory into practice-best practice management for osteoarthritis. In symposium proceedings: Hill's European symposium on osteoarthritis and joint health. Genova, 25<sup>th</sup> to 27<sup>th</sup> April 2005; 48-53.
- Carmines EG, Zeller RA. Reliability and validity assessment. Ed. Sullivan JL. Sage publications.CA, USA 1979:9-70.
- Cattell RB. *A guide to statistical techniques*. The scientific use of factor analysis in behavioral and life sciences. New York: Plenum Press, 1978; 17-32.
- Caughey DE, Grigor RR, Caughey EB, Young P, Gow PJ, Stewart AW. Perna Canaliculus in the treatment of Rheumatoid Arthritis. *Eur J Rheum Inflamm* 1983;6:197-200.
- Chalmers HJ, Dykes NL, Lust G, Farese JP, Burton-Wurster NI, Williams AJ, Todhunter RJ. Assessment of bone mineral density of the femoral head in dogs with early osteoarthritis. *Am J Vet Res* 2006;67:796-800.
- Chan PS, Caron JP, Orth MW. Short term gene expression changes in cartilage explants stimulated with IL-1 $\beta$  plus glucosamine and chondroitin sulfate. *J Rheum* 2006;33:1329-1340.
- Cho SH, Jung YB, Seong SC, Park HB, Byun KY, Lee DC, Song EK, Son JH. Clinical efficacy and safety of Lyprinol, a patented extract from New Zealand green-lipped mussel (*Perna canaliculus*) in patients with osteoarthritis of the hip and knee:a multicenter 2-month clinical trial. *Allerg Immunol (Paris)* 2003;35:212-216.
- Cho ZH, Oleson TD, Alimi D, Niemtow RC. Acupuncture: The search for biologic evidence with functional magnetic resonance imaging and positron emission tomography techniques. *J Altern Complement Med* 2002a;8:399-401.
- Cho ZH, Son YD, Han JY, Wong EK, Kang CK, Kim KY, Kim HK, Lee BY, Yim YK, Kim KH. fMRI neurophysiological evidence of acupuncture mechanisms. *Med Acupuncture* 2002b;14:16-22.
- Cho ZH, Wong EK, Fallon J. In: *Neuro-Acupuncture. Vol. I. Neuroscience Basics*. Q-puncture, Inc. Los Angeles, CA, USA. 2001.

- Clark DM. Current concepts in the treatment of degenerative joint disease. *Compend Contin Educ Pract Vet* 1991;13:1439-1446.
- Clegg DO, Reda DJ, Harris CL, Klein MA, O'Dell JR, Hooper MM et al. Glucosamine, Chondroitin Sulfate, and the Two in Combination for Painful Knee Osteoarthritis. *New England J Med* 2006;354:795-808.
- Cleland LG, French J, Betts HW, Murphy G, Elliot M. Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. *J Rheumatol* 1988;15:1471-1475.
- Cobb CS, Ernst E. Systematic review of a marine nutraceutical supplement in clinical trials for arthritis: the effectiveness of the New Zealand green-lipped mussel *Perna canaliculus*. *Clin Rheumatol* 2005;25:275-284.
- Cohen J. *Statistical power analysis for the behavioral sciences*. 2<sup>nd</sup> ed. Hillsdale, New Jersey: Lawrence Erlbaum Assoc, 1988:1-552.
- Conzemius MG, Hill CM, Sammarco JL, Perkowski SZ. Correlation between subjective and objective measures used to determine severity of postoperative pain in dogs. *J Am Vet Med Assoc* 1997;210:1619-1622.
- Cook JL. Outcomes-based patient care in veterinary surgery: What is an outcome measure? *Vet Surg* 2007;36:187-188.
- Creamer P, Hochberg MC. Osteoarthritis. *Lancet* 1997;350:503-509.
- Crolle G, DiEste E. Glucosamine sulphate for the management of arthrosis. *Curr Ther Res* 1980;7:104-109.
- Cronbach LJ. Coefficient  $\alpha$  and the internal structure of tests. *Psychometrika* 1951;16:297-334.
- Curtis CL, Harwood JL, Dent CM, Caterson B. Biological basis for the benefit of nutraceutical supplementation in arthritis. *Drug Discov Today* 2004;9:165-172.
- D'Ambrosio E, Casa B, Bompani G. et al. Glucosamine sulphate: a controlled clinical investigation in arthrosis. *Pharmatherapeutica* 1981;2:5048.
- Danscher G. Localization of gold in biological tissue. A photochemical method for light and electronmicroscopy. *Histochem* 1981;71:81-88.
- Danscher G. In vivo liberation of gold ions from gold implants. Autometallographic tracing of gold in cells adjacent to metallic gold. *Histochem Cell Biol* 2002;117:447-452.
- Danscher G., Stoltenberg, m. "AUTOMETALLOGRAPHY (AMG). Solver enhancement of quantum dots resulting from (1) metabolism of toxic metals in animals and humans, (2) in vivo, in vitro and immersion created zinc-sulphur/ zinc-selenium nanocrystals, (3) metal ions liberated from metal implants and particles. *Prog Histochem Cytochem* 2006;41:57-139.
- Daubs BM, Markel MD, Manley PA.. Histomorphometric analysis of articular cartilage, zone of calcified cartilage, and subchondral bone plate in femoral heads from clinically normal dogs and dogs with moderate or severe osteoarthritis. *Am J Vet Res* 2006;67:1719-1724.
- Debreceni L. Chemical releases associated with acupuncture and electric stimulation. *Crit Rev Phys Rehabil Med* 1993;5:247-275.

- DeHaan JJ, Goring RL, Beale BS. Evaluation of polysulfated glycosaminoglycan for the treatment of hip dysplasia in dogs. *Vet Surg* 1994;23:177-181.
- Desborough JP. The stress response to trauma and surgery. *Br J Anaesth* 2000;85:109-117.
- DeVellis RF. *Scale development - Theory and applications*. 2<sup>nd</sup> ed. Applied social research methods series, volume 26. CA, USA: SAGE publications, 2003:1-137.
- Dobenecker B, Beetz, Kienzle E. A placebo-controlled double-blind study on the effect of nutraceuticals (chondroitin sulfate and mussel extract) in dogs with joint diseases as perceived by their owners. *J Nutr* 2002;132:S1690-1691.
- Dobromylskyj P, Flecknell PA, Lascelles BD, Livingston A, Taylor P, Waterman-Pearson A. Pain assessment. In: *Pain Management in Animals*. Flecknell PA, Waterman-Pearson A, eds. London: W.B.Saunders, 2000:53-79.
- Dorfer L, Moser M, Bahr F, Spindler K, Egartner-Vigl E, Guillen S, Dohr G, Kenner T. A medical report from the stoneage? *Lancet* 1999;354:1023-1025.
- dos Santos AL, Perazzo FF, Cardoso LGV, Carvalho JCT. In vivo study of the anti-inflammatory effect of Rhus Toxicodendron. *Homeopathy* 2007;96:95-101.
- Drovanti A, Bignamini AA, Rovati AL. Therapeutic activity of oral glucosamine sulfate in osteoarthritis: a placebocontrolled doubleblind investigation. *Clin Ther* 1980;3:260-272.
- Durkes TE. Gold bead implantation. In International Veterinary Acupuncture Society (IVAS) Certification Course Program, San Diego, Ca. 1989.
- Durkes TE. Gold bead implants. In *Veterinary Acupuncture - Ancient art to modern medicine*. Schoen A (ed). American Veterinary Publications Inc. CA, USA. 1994:285-289.
- Durkes TE. Gold bead implants. *Probl Vet Med* 1992;4:207-11.
- Durkes, T. E. (1999) Gold bead implantation in small animals. Proceedings of the 25th Annual International Congress of Veterinary Acupuncture, Lexington, KY, USA, September 1999:1-5.
- Edge-Hughes L, Nicholson H. Canine treatment and rehabilitation. In: *Animal physiotherapy - Assessment, treatment and rehabilitation of animals*. McGowan L, Goff L, Stubbs N (eds.) Blackwell publishing, Oxford, UK 2007:207-237.
- Empire Rheumatism Council (Research subcommittee). Gold therapy in rheumatoid arthritis: final report of a multi-center controlled trial. *Ann Rheum Dis* 1961;20:315-334.
- Ernst E. A systematic review of systematic reviews of homeopathy. *Br J Clin Pharmacol* 2002;54:577-582.
- Eskinazi D. Homeopathy re-revisited: Is homeopathy compatible with biomedical observations? *Arch Intern Med* 1999;159:1981-1987.
- Farrar JT, Portenoy RK, Berlin JA, Kinman JL, Strom BL. Defining the clinically important difference in pain outcome measures. *Pain* 2000;88:287-294.
- Faulstich A, Lutz H, Hellman K. Comparison of the effect of Zeel ad us vet. In lameness of horses caused by non-infectious arthropathy to the effect of hyaluronic acid. *Practischer Tierarzt* 2006;87.

- FCI - Federation Canine International. Hip Dysplasia and Elbow classification, (brochure) Workshop in Dortmund 14.6 1991.
- FDA (Food and drug administration) US ADE (United States Adverse Drug Experience) reports summary 1998. *Anim Pharm* 1999;435:9.
- Felson DT, Anderson JJ, Naimark A, et al. Obesity and knee osteoarthritis. The Framingham study. *Ann Intern Med* 1988;109:18-24.
- Felson DT, Zhang Y, Anthony JM, et al. Weight loss reduces the risk for symptomatic knee osteoarthritis in women. The Framingham study. *Ann Intern Med* 1992;116:535-559.
- Firth AM, Haldane SL. Development of a scale to evaluate postoperative pain in dogs. *J Am Vet Med Assoc* 1999; 214:651-659.
- Flecknell PA. Animal Pain - An introduction. In: *Pain Management in Animals*. Flecknell PA, Waterman-Pearson A (eds.) W.B. Saunders, London 2000:1-7.
- Fox SM, Johnston SA. Use of carprofen for the treatment of pain and inflammation in dogs. *J Am Vet Med Assoc* 1997;210:1493-1498.
- Fransen M, McConnell S, Bell M. Exercise for osteoarthritis of the hip or knee (Cochrane review). In The Cochrane Library, Issue 4. Chichester, UK: John Wiley & Sons, Ltd. 2003.
- Frass M, Schuster E, Muchitsch I, Duncan J, Gei W, Kozel G, Kastinger-Mayr C, Felleitner AE, Reiter C, Endler C, Oberbaum M. Bias in the trial and reporting of trials of homeopathy: a fundamental breakdown in peer review and standards? *J Altern Complement Med* 2005;11:780-782.
- Gaw AC, Chang LW, Shaw L-C. Efficacy of acupuncture on osteoarthritic pain – a controlled, double-blind study. *N Engl J Med* 1975;293:375-378.
- Gelse K, Aigner T, Stöve J, Schneider H. Gene therapy approaches for cartilage injury and osteoarthritis. *Curr Med Chem* 2005;4:265-279.
- Gibson RG., Gibson SLM, Conway V, Chappell D. Perna Canaliculus in the treatment of arthritis. *The Practitioner* 1980; 224:955-960.
- Gibson SLM, Gibson RG. The treatment of arthritis with a lipid extract of Perna Canaliculus: A randomized trial. *Complement Ther Med* 1998;6:122-126.
- Gielen I. Comparison of imaging techniques used in the osteoarthritic joint and their interpretation. In Hill's European symposium on osteoarthritis and joint health. Genova, 25<sup>th</sup>-27<sup>th</sup> April 2005:32-39.
- Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* 2005;434:644-648.
- Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clin Orthop Rel Res* 2004;427:S27-36.
- Gordon WJ, Conzemius MG, Riedesel E, Besancon MF, Evans R, Wilke V, Ritter MJ. The relationship between limb function and radiographic osteoarthrosis in dogs with stifle osteoarthrosis. *Vet Surg* 2003;32:451-454.
- Greenhalgh T. How to read a paper. Statistics for the non-statistician. *Br Med J* 1997;315. (<http://www.bmj.com/archive/7104/7104ed.htm>)

- Guedj D, Weinberger A. Effect of weather conditions on rheumatic patients. *Ann Rheum Dis* 1990;49:158-159.
- Ha H. Naloxone reversal of acupuncture analgesia in the monkey. *Exp Neurol* 1981;73:298-303.
- Halpern GM. *The inflammation revolution: A natural solution for arthritis, asthma and other inflammatory disorders*. Square one publishing, Inc. NY, USA. 2006.
- Hamunen K, Kalso E. A systematic review of trial methodology, using the placebo groups of randomized controlled trials in paediatric post-operative pain. *Pain* 2005;116:146-158.
- Hardie EM. Recognition of pain behavior in animals. In: *Animal pain*. Hellebrekers LJ (ed). Van der Wees, Utrecht, The Netherlands, 2000:51-69.
- Hauptman JG, Richter MA, Wood SL, Nachreiner RF. Effects of anesthesia, surgery, and intravenous administration of fluids on plasma antidiuretic hormone concentrations in healthy dogs. *Am J Vet Res* 2000;61:1273-1276.
- Heel. In: *Biotherapeutic Index – Ordinatio Antihomotoxica et Materia Medica*, 5<sup>th</sup> revised English edition, Baden-Baden, Germany. Biologische Heilmittel Heel GmbH: 2000; pp 9-23, 90-93, 434-436.
- Heinegård D, Sommarin Y. Proteoglycans: an overview. In: *Methods of enzymology, structural and contractile proteins, Part D, extracellular matrix* Cunningham LW (ed). Academic, Orlando, FL, USA 1987;144:305-319.
- Hektoen L. Review of the current involvement of homeopathy in veterinary practice and research. *Vet Rec* 2005;157:224-229.
- Helms JM. *Acupuncture energetics: A clinical approach for physicians*. Medical Acupuncture Publishers, Berkeley, CA, USA. 1995.
- Hennessy MB, Davis HN, Williams MT, Mellott C, Douglas CW. Plasma cortisol levels of dogs at a county animal shelter. *Physiol Behav* 1997; 62:485-490.
- Hielm-Bjorkman A. A two year follow-up after the controlled double-blind trial of hip-gold-bead implantations at Helsinki University. In: The proceedings of the 29th IVAS yearly congress, Brazil, August 2003:377-378.
- Hielm-Björkman A, Reunanen V, Meri P, Tulamo RM: Panax Ginseng in combination with brewers' yeast (Gerivet®) as a stimulant for geriatric dogs: a controlled-randomized blinded study. *J Vet Pharmacol Therap* 2007;30:295-304.
- Holton L, Reid J, Scott EM, Pawson P, Nolan AM. Development of a behaviour-based scale to measure acute pain in dogs. *Vet Rec* 2001;148:525-531.
- Holton L, Scott EM, Nolan AM, Reid J, Welsh E, Flaherty D. Comparison of three methods used for assessment of pain in dogs. *J Am Vet Med Assoc* 1998;212:61-66.
- Holtsinger RH, Parker RB, Beale BS, Friedman RL. The therapeutic efficacy of carprofen (Rimadyl-V™) in 209 clinical cases of canine degenerative joint disease. *Vet Comp Orthop Traumatol* 1992;5:140-144.
- Hoopwood V, Lavesey M, Mokone S (eds). *Acupuncture and related techniques in physical therapy*. Churchill Livingstone, NY, USA. 1997.
- Hudson JT, Slater MR, Taylor L, Scott HM, Kerwin SC. Assessing repeatability and validity of a visual analogue scale questionnaire for the use in assessing pain and

- lameness in dogs. *Am J Vet Res* 2004;65:1634-1643.
- Huijjer Abu-Saad HH, Uiterwijk M. Pain in children with juvenile rheumatoid arthritis: a descriptive study. *Pediatr Res* 1995;38:194-197.
- Hulme J, Robinson V, DeBie R, Wells G, Judd M, Tugwell P. Electromagnetic fields for the treatment of osteoarthritis (Cochrane Review). In: *The Cochrane Library*, Issue 4. Chinchester, UK: John Wiley & Sons, Ltd. 2003.
- Huskisson EC, Scott J, Bryans R. Seatone is ineffective in rheumatoid arthritis. *Br Med J* 1981;282:1358-1359.
- Impellizeri JA, Tetrick MA, Muir P. Effect of weight reduction on clinical signs of lameness in dogs with hip osteoarthritis. *J Am Vet Med Assoc* 2000;216:1089-1091.
- Innes JF, Barr AR. Can owners assess outcome following treatment of canine cruciate ligament deficiency? *J Small Anim Pract* 1998;39:373-378.
- Innes JF, Barr ARS, Sharif M. Efficacy of oral calcium pentosan polysulphate for the treatment of osteoarthritis of the canine stifle joint secondary to cranial cruciate ligament deficiency. *Vet Rec* 2000;146:433-437.
- Innes JF, Fuller CJ, Grover ER, Kelly AL, Burn JF. Randomized, double-blind, placebo-controlled parallel group study of P54FP for the treatment of dogs with osteoarthritis. *Vet Rec* 2003;152:457-460.
- Innes JF, Little CB, Hughes CE, Caterson B. Products resulting from cleavage of the interglobular domain of aggrecan in samples of synovial fluid collected from dogs with early- and late-stage osteoarthritis. *Am J Vet Res*. 2005;66:1679-85.
- Innes J. Canine osteoarthritis - initiating factors. In Hill's European symposium on osteoarthritis and joint health. Genova, 25<sup>th</sup>-27<sup>th</sup> April 2005:6-13.
- Jaeger GT, Larsen S, Soli N, Moe L. Double-blind, placebo-controlled trial of the pain-relieving effects of the implantation of gold beads into dogs with hip dysplasia. *Vet Rec* 2006;158:722-726.
- Jaeger GT, Larsen S, Soli N, Moe L. Two years follow-up study of the pain relieving effect of gold bead implantation in dogs with hip-joint arthritis. *Acta Vet Scand* 2007;49:9 doi:10.1186/1751-0147-49-9.
- Jäggi R, Wurgler U, Grandjean F, Weiser M. Dual inhibition of 5-lipoxygenase/cyclooxygenase by a reconstituted homeopathic remedy; possible explanation for the clinical efficacy and favourable gastrointestinal tolerability. *Inflamm Res* 2004;53:150-157.
- Janssens LAA, Schollaert W, Van Den Bosch E, Van Herbergen D. Anatomical variability of some major acupuncture points in the dog. *Am J Acup* 1987;16:263-267.
- Janssens LAA, Still J. *Acupuncture points and meridians in the dog*. Van Wilderode, Zaventem, Belgium. 1995.
- Janssens LAA. Acupuncture therapy for the treatment of chronic osteoarthritis in dogs: A review of 61 cases. *Vet Med Small Anim Clin* 1976;71:465-468.
- Jevens DJ, DeCamp CE, Hauptman J, Braden TD, Richter M, Robinson R. Use of force-plate analysis of gait to compare two surgical techniques for treatment of cranial cruciate ligament rupture in dogs. *Am J Vet Res* 1996;57:389-393.

- Jevens DJ, Hauptman J, DeCamp CE, Budsberg SC, Soutas-Little RW. Contributions to variance in force-plate analysis of gait in dogs. *Am J Vet Res* 1993;54:612-615.
- Johnson KA, Hulse DA, Hart RC, et al. Effects of an orally administered mixture of chondroitin sulphate, glucosamine hydrochloride and manganese ascorbate on synovial fluid chondroitin sulphate 3B3 and 7D4 epitope in a canine cruciate ligament transection model of osteoarthritis. *Osteoarthritis Cartilage* 2001;9:14-21.
- Johnston CC. Psychometric issues in the measurements of pain. In *Progress in pain research and management*. Finley GA, McGrath PJ (Eds.) IASP Press, Seattle 1998:5-20.
- Kaiser HF. The application of electronic computers to factor analysis. *Educ Psychol Measurements* 1960;20:141-151.
- Kapatkin AS, Arbittier G, Kass PH, Gilley RS, Smith GK. Kinetic gait analysis of healthy dogs on two different surfaces. *Vet Surg* 2007b;36:605-608.
- Kapatkin AS, Mayhew PH, Smith GK. Canine Hip Dysplasia: Evidence-Based Treatment. *Compend Contin Educ Pract Vet* 2002;24:590-599.
- Kapatkin AS. Outcome-based medicine and its applications in clinical surgical practice. *Vet Surg* 2007a;36:515-518.
- Kealy RD, Lawler DF, Ballam JM, Lust G, Biery DN, Smith GK, Mantz SL. Evaluation of the effect of limited food consumption on radiographic evidence of osteoarthritis in dogs. *J Am Vet Med Assoc* 2000;217:1678-1680.
- Kealy RD, Lawler DF, Ballam JM, Olsson SE, Monti KL et al. Five-year longitudinal study on limited food consumption and development of osteoarthritis in coxofemoral joints of dogs. *J Am Vet Med Assoc* 1997;210:222-225.
- Kealy RD, Olsson SE, Monti KL et al. Effects of limited food consumption on the incidence of hip dysplasia in growing dogs. *J Am Vet Med Assoc* 1992;201:857-863.
- Kendall DE. A model of acupuncture. Parts I and II. *Am J Acupunct* 1989;17:343-360
- Kiene H, Kienle GS, Schon-Angerer T. Bias in meta-analysis. *Homeopathy* 2006;95:54.
- Kleijnen J, Knipschild P, Ter Riet G. Clinical trials of homeopathy. *Br Med J* 1991;302:316-323.
- Klide AM. Use of Acupuncture for the Control of Chronic Pain and for Surgical Analgesia. In *Animal Pain*. New York, Churchill Livingstone. 1992:63-79.
- Kline P. Psychometric theory and method. In: *The handbook of psychological testing*. London: Routledge, 1993:5-170.
- Klitsgaard J. Gold Implants; practical experiences with 400 hip dysplasia cases in the dog. In: Proceedings of the 22<sup>nd</sup> Annual International Congress of Veterinary Acupuncture, Switzerland 1996:1-5.
- Klitsgaard J. Guldakupunktur. *Dansk Veterinærtidsskrift* 1995;78:125-127.
- Korthauer W, Torre J. Treatment of deforming arthropathy in working dogs with "canosan", a new glycosaminoglycan preparation. *Kleintierpraxis* 1992;37:467-478.
- Kothbauer, O. Implantation of gold particles into acupuncture points for treatment of painful hip processes in dogs: three cases (in German). *Wien Tierärztl Mschr* 1997;84:47-52.

- Kothbauer, O. Anatomical verification of acupuncture in cattle: a review. *Vet. Med. Austria / Wien Tierärztl Mschr* 2004;91:4-13.
- Kremer JM. Clinical studies on omega-3 fatty acid supplementation in patients who have rheumatoid arthritis. *Rheum Dis Clin North Am* 1991;17:391-402.
- Larsen A, Stoltenberg M, Danscher G. In vitro liberation of charged gold atoms: autometallographic tracing of gold ions released by macrophages grown on metallic gold surfaces. *Histochem Cell Biol* 2007;128:1-6.
- Lascelles BDX, Main DCJ. Surgical trauma and chronically painful conditions – within our comfort level but beyond theirs? *J Am Vet Med Assoc* 2002;221:215-222.
- Lawson BR, Belkowski SM, Whitesides JE, Davis P, Lawson JW. Immunomodulation of murine collagen-induced arthritis by N, N-dimethylglycine and a preparation of *Perna canaliculus*. *BMC Complement Altern Med* 2007;7:20 doi:10.1186/1472-6882-7-20.
- LeBars D, Dickenson AH, Besson JM. Diffuse noxious inhibitory controls. I. Effects on dorsal horn convergent neurons in the rat. II. Lack of effect on nonconvergent neurons, supraspinal involvement and theoretical implications. *Pain* 1979;6:283-327.
- Lees P. Clinical pharmacology of NSAIDs: Current status and future prospects. In symposium proceedings: Hill's European symposium on osteoarthritis and joint health. Genova, 25<sup>th</sup> to 27<sup>th</sup> April 2005;40-47.
- Leighton EA. Genetics of canine hip dysplasia. *J Am Vet Med Assoc* 1997;210:1474-1479.
- Ley SJ, Livingstone A, Waterman AE. Effects of chronic lameness on the concentrations of cortisol, prolactin and vasopressin in the plasma of sheep. *Vet Rec* 1991;129:45-47.
- Ley SJ, Livingstone A, Waterman AE. Effects of clinically occurring chronic lameness in sheep on the concentrations of plasma noradrenaline and adrenaline. *Res Vet Sci* 1992;53:122-125.
- Ley SJ, Waterman AE, Livingstone A, Parkinson TJ. Effect of chronic pain associated with lameness on plasma cortisol concentrations in sheep: a field study. *Res Vet Sci* 1994;57:332-335.
- Li B, Aspden RM. Composition and mechanical properties of cancellous bone from the femoral head of patients with osteoporosis or osteoarthritis. *J Bone Miner Res* 1997;12:641-651.
- Linde K, Clausius N, Ramirez G, Melchart D, Eitel F, Hedges LV, Jonas WB. Are the clinical effects of homeopathy placebo effects? A meta-analysis of placebo-controlled trials. *Lancet* 1997;350:834-843.
- Linde K, Scholz M, Ramirez G, Clausius N, Melchart D, Jonas WB. Impact of study quality on outcome in placebo-controlled trials of homeopathy. *J Clin Epidemiol* 1999;52:631-636.
- Little CV, Parsons T, Logan S. Herbal therapy for treating osteoarthritis (Cochrane Review). In: *The Cochrane Library*, Issue 4. Chichester, UK: John Wiley & Sons, Ltd. 2003.

- Liu YC, Tseng FL, Feng JY, Lam CF, Tsai YC. Pain intensity and barometric pressure closely correlate in Southern Taiwan. *Acta Anaesthesiol Taiwan* 2006;44:147-152.
- Lüdtke R, Hacke D. On the effectiveness of the homeopathic remedy Arnica Montana. *Wien Med Wochenschr* 2005;155:482-490.
- Ma YT, Ma M, Cho ZH. Biomedical acupuncture for pain management – an integrative approach. Elsevier. Missouri, USA. 2005:1-16, 24-51.
- MacPhail CM, Lappin MR, Meyer DJ, Smith SG, Webster CRL, Armstrong PJ. Hepatocellular toxicosis associated with administration of carprofen in 21 dogs. *J Am Vet Med Assoc* 1998;212:1895-1901.
- MacPherson H, White A, Cummings M, JobstK, Rose K, Niemtzw R. Standards for reporting interventions in controlled trials of acupuncture – The STRICTA recommendations. *Acupunct Med* 2002;20:22-25.
- Macrides TA, Treschow AP, Kalafatis N, Wright PFA. The anti-inflammatory effects of Omega-3 Tetraenoic fatty acids isolated from a lipid extract from the New Zealand Green-Lipped Mussel. In: Proceedings of the 88<sup>th</sup> American Oil Chemists Society Annual Meeting: May 1997; Seattle 1997.
- Mani S, Lawson JW. In vitro modulation of inflammatory cytokine and IgG levels by extracts of *Perna canaliculus*. *BMC complement Altern Med* 2006;6:1 doi:10.1186/1472-6882-6-1.
- Maronna U, Weiser M, Klein P. Orale Behandlung der Gonoarthrose mit Zeel comp / Comparison of the efficacy and tolerance of Zeel comp. and Diclofenac for the treatment of gonoarthrosis: Results of a doubleblind Equivalence study. *Orthop Prax* 2000;36:285-291.
- Mascolo N, Autore G, Capasso F, Menghini A, Fasulo MP. Biological screening of Italian medicinal plants for anti-inflammatory activity. *Phytother Res* 1987;1:28-31.
- May SA. Degenerative joint disease. In: *Manual of small animal arthrology*. Houlton J, Collinson R (eds). British Small Animal Veterinary Association, Cheltenham:1994:62-71.
- McCarthy G, O'Donovan J, Jones B, McAllister H, Seed M, Mooney C. Randomized double-blind, positive-controlled trial to assess the efficacy of glucosamine/chondroitin sulphate for the treatment of dogs with osteoarthritis *Vet J* 2007;174:54-61.
- McCarthy RN, Jeffcott LB, Clarke IJ. Preliminary studies on the use of plasma beta-endorphine in horses as an indicator of stress and pain. *J Equine Vet Sci* 1993;3:216-219.
- McManus CA, Schnyer RN, Kong J, Nguyen LT, Nam BH, Goldman R, Stason WB, Kaptchuk TJ. Sham acupuncture devices- practical advice for researchers. *Acupunct Med* 2007;25:36-40.
- Melzack R, Casey KL. Sensory, motivational and central control determinants of pain. In: *The skin senses*. Kenshalo DR (ed). Charles C Thomas Publisher, Springfield, Ill. USA.1968:423-443.
- Melzack R, Wall PD. Pain mechanisms: a new theory. *Science* 1965;150:971.

- Millis D, Levine D. Arthritis management. In: Proceedings 2<sup>nd</sup> International Symposium on rehabilitation and physical therapy in veterinary medicine. Knoxville, Tennessee, USA 2002.
- Millis D. Osteoarthritis pathophysiology. In: Proceedings of Eläinlääkäripäivät 12-14 Oct. 2005, Helsinki, Finland. 2005:236-244.
- Millis DL. Assessing and measuring outcomes. In: *Canine rehabilitation & physical therapy*. Saunders, Missouri, USA 2004:211-227.
- Miossec P. An update on the cytokine network in rheumatoid arthritis. *Curr Opin Rheumatol* 2004;16:218-222.
- Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *Lancet* 2001;357:1191-1194.
- Montgomery R. How I treat canine Hip Dysplasia. *Compend Contin Educ Pract Vet* 1998;20:781-788
- Moore GE, Burkman KD, Carter MN, Peterson MR. Causes of death or reasons for euthanasia in military working dogs: 927 cases (1993-1996) *J Am Vet Med Assoc* 2001;219:1209-214.
- Moreau M, Dupuis J, Bonneau M, Desnoyers M. Clinical evaluation of a neutraceutical, carprofen and meloxicam for the treatment of dogs with osteoarthritis. *Vet Rec* 2003;152:323-329.
- Morreale P, Manopulo R, Galati M, Boccanera L, Saponati G, Bocchi L. Comparison of the anti-inflammatory efficacy of chondroitin sulfate and diclofenac sodium in patients with knee osteoarthritis. *J Rheumatol* 1996;23:1385-1391.
- Morton CM, Reid J, Scott M, Holton LL, Nolan AM. Application of a scaling model to establish and validate an interval level pain scale for assessment of acute pain in dogs. *Am J Vet Res* 2005;66:2154-2166.
- Morton DB, Griffiths PHM. Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. *Vet Rec* 1985;116:431-436.
- Munar A, Gamboa OA, Ortiz NI. Homeopathy for osteoarthritis (Protocol). In: *The Cochrane Library*, Issue 3. Chichester, UK: John Wiley & Sons, Ltd. 2007.
- Nahler G, Metelmann H, Sperber H. Behandlung der Gonoarthrose mit Zeel comp. – Ergebnisse einer randomisierten, kontrollierten klinischen Prüfung im Vergleich zu Hyaluronsäure. *Orthop Prax* 1996;5:354-359 (in German).
- Neumann ST, Braun G, Hellman K, Reinhart E. Behandlung von degenerative Gelenkerkrankungen beim Hund mit Zeel® ad us vet. im Vergleich zu Carprofen. *Kleintiermedizin* 2007;7/8:215-226 (in German).
- NIH consensus conference on Acupuncture. *J Am Med Assoc* 1998;280:1518-1524.
- Nunnally JC. *Psychometric Theory*. 2nd ed. New York: McGraw-Hill, 1978;86-113, 225-325.
- Official Statistics of the Finnish Kennel Club 1988-2007. <http://jalostus.kennelliitto.fi/frmTerveystilastot.aspx?R=122>.

- Oliveira SA, Felson DT, Reed JI, Walker AM. Body weight and the development of incident hand, hip and knee osteoarthritis in women. *Arthritis Rheum* 1995;38:S341.
- Olsewski JM, Lust G, Rendano VT et al. Degenerative joint disease: multiple joint involvement in young and mature dogs. *Am J Vet Res* 1983;44:1300-1308.
- Orlandini A, Rossi M, Setti M. Die Wirksamkeit von Zeel und neue Forschungsmethoden in der Rheumatologie. *Biol Med* 1997;26:164-165.
- Osiri M, Welch V, Brosseau UL, Shea B, McGovan J, Tugwell P. et al. Transcutaneous electrical nerve stimulation for knee osteoarthritis (Cochrane Review). In: *The Cochrane Library*, Issue 4. Chinchester, UK: John Wiley & Sons, Ltd. 2003.
- Park J, White A, Stevinson C, Ernst E, James M. Validating a new non-penetrating sham acupuncture device: two randomised controlled trials. *Acup Med* 2002;20:168-174.
- Pascoe P. Alternative methods for the control of pain. *J Am Vet Med Assoc* 2002;221:222-229.
- Paster ER, La Fond E, Biery DN, Iriye A, Gregor TP, Shofer FS, Smith GK. Estimates of prevalence of hip dysplasia in Golden Retrievers and Rottweilers and the influence of bias on published prevalence figures. *J Am Vet Med Assoc* 2005;226:387-392.
- Paterson C, Dieppe P. Characteristic and incidental (placebo) effects in complex interventions such as acupuncture. *Br Med J* 2005;330:1202-1205.
- Pelletier JP, Martel-Pelletier J, Raynauld JP. Most recent developments in strategies to reduce the progression of structural changes in osteoarthritis: today and tomorrow. *Arthritis Res Ther* 2006;8:206.
- Pelletier JP, Yaron M, Haraoui B, Cohen P, nahir MA, Choquette D, Wigler I, Rosner IA, Beaulieu AD. Efficacy and safety of diacerein in osteoarthritis of the knee: a double-blind, placebo controlled trial by the diacerein study group. *Arthritis Rheum* 2000;43:2339-2348.
- Pendleton A, Arden N, Dougados M, Doherty M, Bannwarth B, Bijlsma JWJ, Cluzeau F, Cooper C, Dieppe PA, Gunther KP, Hauselmann HJ, Herrero-Beaumont G, Kaklamanis PM, et al. EULAR recommendations for the management of knee osteoarthritis. Report of a task force of the standing committee for international clinical studies including therapeutic trials (ESCISIT). *Ann Rheum Dis* 2000;59:936-44.
- Persillin RH, Ziff M. The effect of gold salt on lysosomal enzymes of the peritoneal macrophage. *Arthritis Rheum* 1966;9:57-65.
- Peterson KD, Keefe TJ. Effects of meloxicam on severity of lameness and other clinical signs of osteoarthritis in dogs. *J Am Vet Med Assoc* 2004;225:1056-1060.
- Pipitone V, Ambanelli U, Cervini C, Ligniere GC, Ugo GT, Ferraris M, Marcolongo R, Megale F, Serni U, Trotta F, Martino F. A multicenter, triple-blind study to evaluate galactosaminoglycuronoglycan sulfate versus placebo in patients with femorotibial gonarthrosis. *Curr Ther Res* 1992;52:608-638.
- Pollard B, Guilford WG, Ankenbauer-Perkins KL, Hedderley D. Clinical efficacy and tolerance of an extract of green lipped mussel (*Perna canaliculus*) in

- dogs presumptively diagnosed with degenerative joint disease. *N Z Vet J* 2006;54:114-118.
- Pomeranz B. Scientific basis of acupuncture. In: *Basics of acupuncture*. Stux G, Pomeranz B (Eds) 4<sup>th</sup> ed. Springer. Berlin. 1998:6-47.
- Pond MJ, Nuki G. Experimentally- induced osteoarthritis in the dog. *Ann Rheum Dis* 1973;32:387-388.
- Poy NJ, DeCamp CE, Bennett RL, Hauptman JG. Additional kinematic variables to describe differences in the trot between clinically normal dogs and dogs with hip dysplasia. *Am J Vet Res* 2000;61:974-978.
- Prado Neto JA, Perazzo FF, Cardoso LG, Bonamin LV, Carvalho JC. Action of causticum in inflammatory models. *Homeopathy* 2004;93:12-16.
- Pujalte JM, Llavore EP, Ylescupidéz FR. Double-blind clinical evaluation of oral glucosamine sulphate in the basic treatment of osteoarthritis. *Curr Med Res Opin* 1980;7:110-114.
- Qiu GX, Gao SN, Giacobelli G et al. Efficacy and safety of glucosamine sulfate versus ibuprofen in patients with knee osteoarthritis. *Arzn Forsch* 1998;48:469-474.
- Quinn MM, Keuler NS, Lu Y, Faria MLE, Muir P, Markel MD. Evaluation of agreement between numerical rating scales, visual analogue scoring scales, and force plate gait analysis in dogs. *Vet Surg* 2007;36:360-367.
- Raekallio M, Hielm-Björkman A, Kejonen J, Salonen H, Sankari S: Evaluation of adverse effects of long-term orally administered carprofen in dogs. *J Am Vet Med Assoc* 2006;228,876-880.
- Raekallio M, Taylor PM, Bloomfield M. A comparison of methods for evaluation of pain and distress after orthopaedic surgery in horses. *J Vet Anaesth* 1997;24:17-20.
- Rainsford KD, Whitehouse MW. Gastroprotective and anti-inflammatory properties of green lipped mussel (*perna canaliculus*) preparation. *Arzn Forsch* 1980;30:2128-2132.
- Reckeweg HH. *Homotoxicologie. Ganzheitsschau Einer Synthese der Medizin*. Aurelia Verlag. Baden-Baden 1981.
- Reichelt A, Förster KK, Fischer M et al. Efficacy and safety of intramuscular glucosamine sulfate in osteoarthritis of the knee. *Arzn Forsch* 1994;44:75-80.
- Reichmanis M. Electric correlates of acupuncture points. *IEEE Transactions on Biomedical Engineering Bio Med Eng* 1975;22:533-535.
- Reichmanis M. Laplace plane analysis of impedance on the H meridian. *Am J Chin Med* 1979;7:188-193.
- Reid J, Wiseman L, Nolan AM, Scott EM. Owner reported behaviour changes associated with chronic pain in dogs. In: Proceedings of the 7<sup>th</sup> world congress of veterinary anaesthesia. Berne Sep. 20-23,2000:126.
- Revill SI, Robinson JO, Rosen M, Hogg MIJ. The reliability of a linear analogue for evaluating pain. *Anaesthesia* 1976; 31:1191-1198.
- Riggs CM, DeCamp CE, Soutal-Little RW, Braden TD, Richter MA. Effects of subject velocity on force-plate measured ground reaction forces in healthy Greyhounds at the trot. *Am J Vet Res* 1993;54:1523-1526.

- Rindone RP. Randomized controlled trial of glucosamine for treating osteoarthritis of the knee. *West J Med* 2000;172:91-94.
- Rita H, Ekholm P. Showing similarity of results given by two methods: A commentary. *Environ Pollut* 2007;145:383-386.
- Robertson SA. What is pain? *J Am Vet Med Assoc* 2002;221:202-205.
- Roush JK, McLaughlin RM. Effects of subject stance time and velocity on ground reaction forces in clinically normal Greyhounds at the walk. *Am J Vet Res* 1994;55:1672-1676.
- Rumph PF, Steiss JE, Montgomery RD. Effects on selection and habituation on vertical ground reaction force in Greyhounds. *Am J Vet Res* 1997;58:1206-1208.
- Rumph PF, Steiss JE, West MS. Interday variation in vertical ground reaction force in clinically normal Greyhounds at the trot. *Am J Vet Res* 1999;60:679-683.
- Rutherford KMD. Assessing pain in animals. *Animal Welfare* 2002;11:31-53.
- Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An Introduction: Cell biology of osteoarthritis. *Arthritis Res* 2001;3:107-113.
- Sanford J, Ewbank R, Molony V, Tavernor WD, Uvarov O. Working party of the Association of the Veterinary Teachers and Research Workers: Guidelines for the recognition and assessment of pain in animals. *Vet Rec* 1986;118:334-338.
- Sanford J. Guidelines for detection and assessment of pain and distress in experimental animals. In: *Animal Pain*. Short, C.E. and Van Poznac, A. (eds.). Churchill Livingstone, New York 1992;515-524.
- Sargent J, Liebman R. Childhood chronic illness: Issues for psychotherapists. *Community Ment Hlt J* 1985;21(4):294-311.
- Scheinin H. Kliinisen tutkimuksen karikoita - riittääkö potilasmäärä? *Finnanest* 1997;30:245-250 (in Finnish).
- Schneiderman LJ. The ethics of alternative medicine. In: *Science meets alternative medicine*. Sampson W, Vaughn L (eds). Prometheus books. NY,USA. 2000:211.
- Schoen A. Acupuncture for musculoskeletal disorders. In: *Veterinary Acupuncture-Ancient art to modern mystery*. 2<sup>nd</sup> Ed., Schoen A. (ed) Mosby, Missouri, USA. 2001.
- Schuirman DJ. A comparison of two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J Pharmacokinet Biopharm* 1987;15:657-680.
- Schultze E. Arthrosen und Goldimplantate. *Kleintiermedizin* 1999;6:240-244 (in German).
- Schultze E. Goldakupunktur oder Goldimplantate?- Nur ein schematisches problem? *Z Ganzheitl Tiermed* 2001;15:139-140 (in German).
- Schultze E. Goldimplantate in der Schmerztherapie. *Z Ganzheitl Tiermed* 1998;12:103-105 (in German).
- Schulz KS, Cook JL, Kapatkin AS, Brown DC. Evidence-Based surgery: Time for change. *Vet Surg* 2006;35:697-699.
- Senikar I, Giacchetti C, Zanolio G. Pharmacokinetics of glucosamine in the dog and in man. *Artzn Forsch* 1986;36:728-735.

- Servet E, Biourge V, Marniquet P. Dietary Intervention can improve clinical signs in osteoarthritic dogs. *J Nutr* 2006;136:S1995-1997.
- Shang A, Huwiler-Muntener K, Nartey L, Juni P, Dörig S, Sterne JAC, Pewsner D, Egger M. Are the clinical effects of homeopathy placebo effects? Comparative study of placebo-controlled trials of homeopathy and allopathy. *Lancet* 2005;366:726-732.
- Shott S. Study design. *J Am Vet Med Assoc* 1990;197:1142-1144.
- Sibley JT. Weather and arthritis symptoms. *J Rheumatol* 1985;12:707-710.
- Siegel S. *Nonparametric statistics for the behavioral sciences*. Tokyo: McGraw-Hill book company, inc. Kôgakusha company, ltd. 1956:25-34, 195-239.
- Slocum B, Slocum DS. Hip - Diagnostic Tests. In: *Current Techniques in Small Animal Surgery*. Bojrab MJ (ed). Williams & Wilkins, Baltimore, USA. 1998:1127-1145.
- Smith GK, McKelvie PJ. Current concepts in the diagnosis of canine hip dysplasia. In: *Kirk's current vet therapy XII - small animal practice*. Bonagura JD (ed). Saunders co. PA, USA 1995:1180-1188.
- Smith GK. Advances in diagnosing canine hip dysplasia. *J Am Vet Med Assoc* 1997;210(10):1451-1457.
- Smith GK, Paster ER, Powers MY, Lawler DF, Biery DN, Shofer FS, McKelvie PJ, Kealy RD. Lifelong diet restriction and radiographic evidence of osteoarthritis of the hip joint in dogs. *J Am Vet Med Assoc* 2006;229:690-693.
- Stancikova M, Bfly M, Svik K, Metelmann HW, Schmolz MW, Istok R, Fano R. Effects of ZEEL Comp. on experimental osteoarthritis in rabbit knee. *Rheuma* 1999a;13:101-108.
- Stancikova M. Hemmung der Leukozytenelastase-Aktivität in vitro mit Zeel® T, Zeel® comp. und ihren verschiedenen potenzierten Bestandteilen. *Biol Med* 1999b;2:83-84 (in German).
- Steiss JE. The neurophysiologic basis of acupuncture. In: *Veterinary Acupuncture – ancient art to modern medicine* (2<sup>nd</sup> edn.). Schoen (ed.) Mosby Inc., Missouri, USA. 2001:27-46.
- Stoker AM, Cook JL, Kuroki K, Fox DB. Site-specific analysis of gene expression in early osteoarthritis using the Pond-Nuki model in dogs. *J Orthop Surg Res* 2006;1:8. doi:10.1186/1749-799X-1-8.
- Streiner DL, Norman GR. *Health Measurement Scales. A Practical Guide to their Development and Use*. 2nd ed. New York: Oxford University Press, 1995:4-161.
- Streiner DL. Research methods in psychiatry. A checklist for evaluating the usefulness of rating scales. *Can J Psychiatry* 1993;38:140-148.
- Strusberg I, Mendelberg RC, Serra HA, Strusberg AM. Influence of weather conditions on rheumatic pain. *J Rheumatol*. 2002;29:335-338.
- Tabachnick BG, Fidell LS. Principal components and factor analysis. In: *Using Multivariate Statistics*. 5<sup>th</sup> ed. Boston: Allyn and Bacon 2007;607-675.
- Tano CA, Cockshutt JR, Dobson H, Miller CW, Holmberg DL, Taves CL. Force plate analysis of dogs with bilateral hip dysplasia treated with a unilateral triple pelvic osteotomy: a long-term review of cases. *Vet Comp Orthop Traumatol* 1998;11:85-93.

- Technyflex/Lyproflex test certificate 2002 [http://www.naturalhealthuk.co.uk/english/technyflex\\_canine.html](http://www.naturalhealthuk.co.uk/english/technyflex_canine.html)
- terRiet G, Kleijnen J, Knipschild P. Acupuncture and chronic pain: a criteria-based meta-analysis. *J Clin Epid* 1990;43:1191-1199.
- Theyse LFH, Hazewinkel HA, Van Den Brom WE. Force plate analyses before and after surgical treatment of unilateral fragmented coronoid process. *Vet Comp Orthop Trauma* 2000;13:135-140.
- Thomas M, Eriksson SV, Lundeberg T. A Comparative Study of Diazepam and Acupuncture in patients with Osteoarthritis Pain: A Placebo Controlled Study. *Am J Chin Med* 1991;14:95-100.
- Thoresen A. A "new" method of placing gold implants to treat hip dysplasia in the dog, description and evaluation of 50 cases 1990-1996. In: Proceedings of the 22<sup>nd</sup> Annual International Congress on Veterinary Acupuncture. 1996:1-5.
- Towheed TE, Anastassiades TP, Shea B, Houpt J, Welch V, Hochberg MC. Glucosamine therapy for treating osteoarthritis (Cochrane Review). In: *The Cochrane Library*, Issue 4. Chichester, UK: John Wiley & Sons, Ltd. 2003.
- Traber KE, Okamoto H, Kurono C, Baba M, Saliou C, Soji T, Packer L, Okamoto T. Anti-rheumatic compound aurothioglucose inhibits tumor necrosis factor- $\alpha$ -induced HIV-1 replication in latently infected OM10.1 and Ach2 cells. *Int Immunol* 1999;11:143-150.
- Trumble TN, Billingham RC, McIlwraith CW. Correlation of prostaglandin E2 concentrations in synovial fluid with ground reaction forces and clinical variables for pain or inflammation in dogs with osteoarthritis induced by transection of the cranial cruciate ligament. *Am J Vet Res* 2004;65:1269-1275.
- Trumble TN, Billingham RC, Bendele AM, McIlwraith CW. Evaluation of changes in vertical ground reaction forces as indicators of meniscal damage after transection of the cranial cruciate ligament in dogs. *Am J Vet Res* 2005;66:156-163.
- Tunon H, Olavsdotter C, Bohlin L. Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. *J Ethnopharmacology* 1995;48:61-76.
- Turk DC, Dworkin RH. What should be the core outcomes in chronic pain clinical trials? *Arthritis Res Ther* 2004;6:151-154.
- Uebelhart D, Thonar EJ, Delmas PD, Chantraine A, Vignon E. Effects of oral chondroitin sulfate on the progression of knee osteoarthritis: a pilot study. *Osteoarthr Cartil* 1998;6A:S39-46.
- Vaillancourt JP, Martineau G, Morrow M, Marsh W, Robinson A. Construction of questionnaires and their use in veterinary medicine. In Proceedings Soc Vet Epidemiol Prevent Med 1991:94-106.
- Väisänen M, Raekallio M, Kuusela E, Huttunen P, Leppäluoto J, Kirves P, Vainio O. Evaluation of the perioperative stress response in dogs administered medetomidine or acepromazine as a part of the preanaesthetic medication. *Am J Vet Res* 2002;63:969-975.

- Väisänen M, Oksanen H, Vainio O. Postoperative signs in 96 dogs undergoing soft tissue surgery. *Vet Rec* 2004;155:729-733.
- Valentiner U, Weiser M, Moll I, Schumacher U. The effect of Homeopathic Plant Extract Solutions on the Cell proliferation of Human Cutaneous Fibroblasts in vitro. *Forsch Komplement Med Klass Nat Heilkd* 2003;10:122-127.
- van Dijk M, Koot HM, Huijter Abu Saad H, Tibboel D, Passchier J. Observational visual analog scale in pediatric pain assessment: Useful tool or good riddance? *Clin J Pain* 2002;18:310-316.
- Varni JW, Thompson KL, Hanson V. The Varni/Thompson pediatric pain questionnaire: I. Chronic musculoskeletal pain in juvenile rheumatoid arthritis. *Pain* 1987;28:27-38.
- Vas J, Mendez C, Perea-Milla E, Vega E, Panadero MD, Leon JM, Borge MA, Gaspar O, Sanchez-Rodrigues F, Aguilar I, Jurado R. Acupuncture as a complementary therapy to the pharmacological treatment of osteoarthritis of the knee: Randomized controlled trial. *Br Med J* 2004. doi:10.1136/bmj.38238.601447.3A
- Vasseur PB, Johnson AL, Budsberg SC, Lincoln JD, Toombs JP, Whitehair JG, Lentz EL. Randomized, controlled trial of the efficacy of carprofen, a nonsteroidal anti-inflammatory drug, in the treatment of osteoarthritis in dogs. *J Am Vet Med Assoc* 1995; 206:807-811.
- Vaughan-Scott T, Taylor JH. The pathophysiology and medical management of canine osteoarthritis. *J S Afr Vet Ass* 1997;68:21-25.
- Veith I. *The Yellow Emperors Classic of Internal Medicine*. University of California Press, Berkeley 1973.
- Verbruggen G, Goemaere S, Veys EM. Chondroitin sulphate: S/DMOAD (structure/disease modifying antiosteoarthritis drug) in the treatment of finger joint OA. *Osteoarthritis Cartilage* 1998;6:S37-38.
- Verges J, Montell E, Tomas E, Cumelles G, Castaneda G, Marti N, Moller I. Weather conditions can influence rheumatic diseases. *Proc West Pharmacol Soc* 2004;47:134-136.
- Volker D, Garg M. Dietary N-3 Fatty Acid Supplementation in Rheumatoid Arthritis- Mechanisms, Clinical Outcomes, Controversies, and Future directions. *J Clin Biochem Nutr* 1996;20:83-97.
- Voss K, Imhof J, Kaestner S, Montavon PM. Forceplate gait analysis at the walk and trot in dogs with low-grade hindlimb lameness. *Vet Comp Orthop Traumatol* 2007;20:299-304.
- Wall PD. Defining "Pain in Animals". In: *Animal Pain*. Short CE, Van Poznac A (eds). Churchill Livingstone, New York. 1992:63-79.
- Watson MC, Brookes ST, Kirwan JR, Faulkner A. Non-aspirin, non-steroidal anti-inflammatory drugs for treating osteoarthritis of the knee (Cochrane Review). In: *The Cochrane Library*, Issue 4. Chichester, UK: John Wiley & Sons, Ltd. 2003.
- Welsh EM, Gettinby G, Nolan AM. Comparison of a visual analog scale and a numerical rating scale for assessment of lameness, using sheep as the model. *Am J Vet Res* 1993;54:976-984.

- Welsh EM, Nolan AM, Reid J. Beneficial effects of administering carprofen before surgery in dogs. *Vet Rec* 1997;141:251-253.
- White AR, Earnst E. A systematic review of randomized controlled trials of acupuncture for neck pain. *Rheumatol* 1999;38:143-147.
- Whitehouse MW, Macrides TA, Kalafatis N, Betts WH, Haynes DR, Broadbent J. The anti-inflammatory activity of a lipid fraction from the New Zealand green lipped mussel. *Inflammopharmacology* 1997;5:237-246.
- WHO. Traditional medicine. Fact sheet 134. 2003:1. <http://www.who.int./mediacentre/factsheets/fs134/en/print.html>
- Wiseman ML, Nolan AM, Reid J, Scott EM. Preliminary study on owner-reported behaviour changes associated with chronic pain in dogs. *Vet Rec* 2001;149:423-424.
- Wiseman Z, Topper R, Oberbaum M, Bentwich Z. Proceedings of 5th GIRI meeting. Paris 1991.
- Wiseman-Orr ML, Nolan AM, Reid J, Scott EM. Development of a questionnaire to measure the effects of chronic pain on health-related quality of life. *Am J Vet Res* 2004;65:1077-1084.
- Wiseman-Orr ML, Scott EM, Reid J, Nolan AM. Validation of a structured questionnaire as an instrument to measure chronic pain in dogs on the basis of effects on health-related quality of life. *Am J Vet Res* 2006;67:1826-1836.
- WMA: World Medical Association declaration of Helsinki – Ethical principles for medical research involving human subjects. Adopted by the 18<sup>th</sup> WMA General Assembly, Helsinki, Finland, June 1964. / Note of clarification 29. Washington, 2002. <http://www.wma.net/e/policy/b3.htm>.
- Wu MT, Hsieh JC, Xiong J, Yang CF, Pan HB, Chen YCI, Tsai G, Rosen BR, Kwong KK. Central nervous pathway for acupuncture stimulation: Localization of processing with functional MR Imaging of the brain – preliminary experience. *Radiology* 1999;212:133-141.
- Wynn SG, Luna SPL, Liu H, Xie H, Nam TC, Chien CH. Global acupuncture research: previously untranslated studies. In: *Veterinary Acupuncture – ancient art to modern medicine* (2<sup>nd</sup> edn.). Schoen A. (ed) Mosby Inc., Missouri, USA 2001:53-77.
- Xie HS et al. A study on location of acupoints in limbs in dog. *Chin J Vet Med* 1994;20:37-40.
- Yamaguchi N, Takahashi T, Sakuma M, Sugita T, Uchikawa K, Sakaiharu S, Kanda T, Arai M, Kawakita K. Acupuncture regulates leukocyte subpopulations in human peripheral blood. *Evid Based Complement Alternat Med* 2007; doi:10.1093/ecam/nel107
- Yang JP, Merin JP, Nakano T, Kato T, Kitade Y, Okamoto T. Inhibition of the DNA-binding activity of NF-kappa B by gold compounds in vitro. *FEBS Lett* 1995;361:89-96.
- Yim YK, Lee H, Hong KE, Kim YI, Lee BR, Son CG, Kim JE. Electro-acupuncture at acupoint ST36 reduces inflammation and regulates immune activity in collagen-induced arthritic mice. *Evid Based Complement Alternat Med* 2007;4:51-57.

- Yoshida S, Kato T, Sakurada S, Kurono C, Yang JP, Matsui N, Soji T, Okamoto T.  
Inhibition of IL-6 and IL-8 induction from cultured rheumatoid synovial  
fibroblasts by treatment with aurothioglucose. *Int Immunol* 1999;11:151-158.
- Zhang LS et al. Resistance measurement of meridian acupoint in healthy animals. *J  
Tradit Chin Vet Med* 1988;1:6-9.
- Zhao YS et al: In: *Veterinary acupuncture*. Agriculture Press, Beijing, China. 1993:272.

