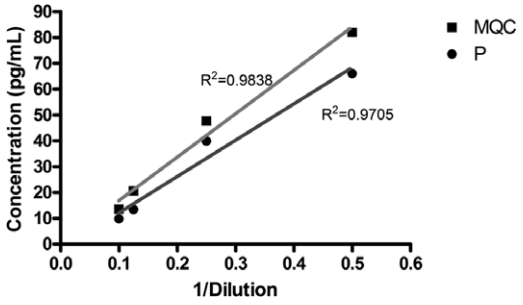

Figure 1. The linearity was analyzed by serially diluting (1:2, 1:4, 1:8, and 1:10)the mixed QC samples (MQC) and the unspiked pooled samples (P). The observed concentration was plotted against the reciprocal of the dilution.

loss in the stability of the CTX II epitope out to 4 freeze/thaw cycles. Differences in biological activity were identified when examining samples from 3 normal rested horses (111.40 ± 33.3 pg/ml), the same horses after 5 months race-training (158.18 ± 111 pg/ml), and those with naturally-occurring OC injuries (38.96 ± 12.91 pg/ml) (Figure 2).

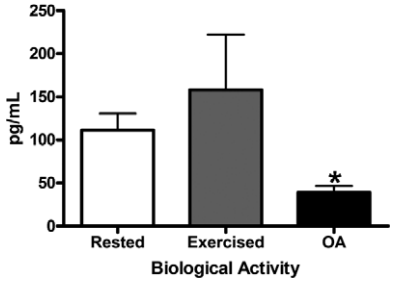

Figure 2. Differences in biological activity identified between samples from 3 horses before (Rested) and after 5 months race-training (Exercised) as well as 3 horses with OA. Significant differences between groups are represented as *P<0.05 using a Kruskal-Wallis test.

Conclusions: The Serum Pre-Clinical Cartilaps® ELISA is a reproducible and valid assay for use with equine serum.

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OSTEOPROTEGERIN AND RECEPTOR ACTIVATOR OF NUCLEAR FACTOR kB LIGAND IN SYNOVIAL FLUID AND SERUM IN PATIENTS WITH PRIMARY KNEE OSTEOARTHRITIS

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Purpose: to evaluate the relationship between OPG and RANKL in synovial fluid and serum in patients with primary knee osteoarthritis and disease severity as it is graded radiologically according to the system of Kallgren and Lawrence. To demonstrate the role of the RANKL/OPG system in the pathophysiology of primary knee osteoarthritis.
Methods: the study population included 37 patients (9 males, 28 females) with mean age 64.9 years (ranging from 50 to 80 years). Synovial fluid was aspirated from the affected joint during surgery where a total knee arthroplasty was performed. Blood samples were obtained from the same patients 1 to 2 hours before surgery. Anteroposterior weight bearing radiographs of the patients' knees were performed and osteoarthritic changes were graded according to the system of Kallgren and Lawrence.
Results: we found that OPG concentration in synovial fluid was significantly higher than in serum (257.5±76.9 vs 49.2±10.1 pmoles/lt, p<0,0001), indicating that the increase of OPG in patients with knee OA is not systemic but rather localized in the affected joint. OPG levels were increased in synovial fluid in relation with the severity of knee OA and were significantly higher in patients with OA grade 4 than in those with grade 1 or 2. RANKL levels were found low in both synovial fluid and serum (1.5±1.0 and 1.0±1.0 pmoles/lt respectively). Serum levels of OPG and RANKL did not correlate with the severity of knee OA
Conclusions: based on the fact that OA is characterized by progressive deterioration of the articular cartilage, the increase of OPG in synovial fluid of individuals with knee OA might reflect a compensatory response by chondrocytes or synovial fibroblasts to destabilization of the coupling between degradation and synthesis of articular cartilage. The increased concentration of OPG might thus serve to protect cartilage rather than be a cause of osteoarthritis.

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COMPARISONS OF ORIGINAL AND AUTHENTIC PHARMACEUTICAL AVOCADO AND SOYA UNSAPONIFIABLES VERSUS SOME ALLEGED NUTRACEUTICAL IMITATIONS BY GAS CHROMATOGRAPHY ANALYSIS

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Purpose: Avocado and Soya (A/S 1:2 w/w) unsaponifiables (ASU), developed and patented by Laboratoires Expanscience, are the components of a medicinal product found in numerous countries against degenerative osteoarthritis. The main ingredients are: 30% tocopherol, 25% sterols and 25% specific active molecules. The originality is based on A/S ratio and Avocado specific modified unsaponifiables obtained by chemical transformation of precursors. This modification by hemisynthesis constitutes one of the Laboratoires Expanscience know-how and is patented.
In vitro published studies showed effects of ASU on the anabolism (increase in the synthesis of collagens, proteoglycans and TGFβ) and catabolism (inhibition of synthesis of MMPs, PGE2, and pro-inflammatory cytokines) of osteoarthritic chondrocytes. Moreover, clinical studies demonstrated the symptomatic effect of ASU combined to a reduction in NSAID administration. The pharmaceutical status requires perfect controls of the extraction process, of the hemisynthesis from precursors to converted molecules and an excellent between-batch reproducibility.
This is ensured by a strict control of the whole plant process: contractual partnerships with local subcontractors, expertise in vegetable lipid chemistry allowing an exact definition of the plant species extracted and the soils together with the application of GMP rules for the whole process from the plant starting material up to the pharmaceutical active ingredient.
Attracted by the scientific renown and success of pharmaceutical ASU, nutraceutical companies have attempted to develop imitations by suggesting more or less clearly a relation between the composition and even the activity of the original pharmaceutical.
Methods: An analytical study of 7 nutraceutical products (for human and veterinary uses) claiming the name ASU has been performed by gas chromatography methodology.
Results: The analytical study of the nutraceutical products versus the original and authentic ASU showed:
– The complete absence of specific molecules which are patented (obtained by transformation of avocado precursors) evaluated by GC assay of silylderivatives on a 5%-phenylmethylpolysiloxane capillary column equipped with a cold on-column injector and a FID detector;
– A content of unsaponifiables of less than 25% measured by a method of the American Oil Chemistry Society N° Ca 6a-40;
– A lower sterol and tocopherol content of less than 15% and 5%

respectively;- Presence of traces of other plant unsaponifiables (like Colza unsaponifiables for example).
Conclusions: These nutraceutical products are therefore completely unrelated to the composition of the original pharmaceutical ASU. The quality of ASU is supported by a global know-how concerning the qualitative management of the plant material from it's cultivation, extraction, hemisynthesis to it's final concentration. The original ASU alone may therefore claim a relationship between the control of biodiversity, characterization of specific structures and its pharmacological and clinical activity in osteoarthritis.

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CARTILAGE BIOMARKERS IN URINE - OBSERVATIONS AFTER MENISCECTOMY

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Purpose: Cartilage biomarkers promise to advance our possibilities to monitor articular cartilage damage and diseases by non-invasive means. Complete medial meniscectomy has been shown to lead to osteoarthritis(OA), in the human as in animal models. In this study, complete medial meniscectomy was used to investigate the potential of the collagenous biomarkers hydroxylysylpyridinoline(HP) and lysylpyridinoline(LP) when measured in urine.
Methods: New Zealand White rabbits (n = 32) had a complete medial meniscectomy of their right knees using a medial parapatellar approach. Open surgery allowed a complete visualisation of the joint structures at all timepoints of the procedure ensuring a safe procedure. Postoperatively, the animals were housed in cages. A group of 32 rabbits were used as unoperated controls. 8 rabbits of each group were sacrificed at 2, 4, 8 and 12 weeks. Macroscopic mapping was performed on the right and left knee joints using the scheme of the International Cartilage Repair Society. Specific areas and sums of areas of all joint sites were analysed for lesions. For microscopic evaluation, 4 μm sections of the central tibial plateaus were stained with H&E and Safranin O. Histologic analysis was performed with a dedicated grading system accounting for pathologic alterations of proteoglycan content, matrix structure, cellularity, tidemark duplication, and osteophyte formation, basically a modified Mankin scheme.
Urine samples were collected during the 24 hrs before surgery and again during 24 hrs before each sacrifice time point. Measurements of hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) were performed with HPLC. Data were normalised to the urinary creatinine. Ratios of the markers were calculated. Statistical calculations were performed with SPSS using e.g. ANOVA, Wilcoxon and Mann Whitney U tests.
Results: No signs of infection were noted during the postoperative course of the animals. Macroscopic lesions advanced with time after meniscectomy. First degenerative changes were already present at 2 weeks after surgery, with an increase in number and size of lesions with time. Histologically, OA-like lesions were present at all 4 examined timepoints.
The overall macroscopic, but not microscopic score correlated inversely with the HP/creatinine ratio. The LP/creatinine ratio was inversely correlated with either one.
Both HP/creatinine and LP/creatinine correlated inversely with the overall histologic score. At the reported timepoints, both, HP/creatinine and LP/creatinine ratios had generally lower mean levels than the control animals. No simple timecourse which might indicate a worsening osteoarthritic disease could be observed with either ratio. This was also true for HP/LP.
Conclusions: Biomarkers have proven valuable in many biological systems and clinical situations eg in gastroenterology, cardiology and oncology. Cartilage biomarkers are still under development coming close to clinical utility. In this study, measurements of HP and LP in the urine of rabbits after complete medial meniscectomy did not show any simple significant postoperative changes. This was true for the respective ratios. Thus we were not able to monitor the development of OA-like lesions of the articular knee joint in a simple manner with these markers. Such somehow negative findings are part of a research development which is still stimulating and will further our detailed knowledge of cartilage and its diseases.

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URINE PRE-CLINICAL CTX II (CARTILAPS®) ASSAY VALIDATION USING EQUINE URINE

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Purpose: The CTX II assay has been developed to examine the type II collagen telopeptide that is released from articular cartilage into the systemic circulation. In human osteoarthritis (OA) patients, the Urine Cartilaps® ELISA has been reported to be useful in the prediction of the progression of OA. Since type II collagen is well conserved across species, this assay was modified by the manufacturers into the pre-clinical assay to allow for a broader measuring range of CTX-II concentrations in non-human urine. Horses are one of the few species in which adequate volumes of paired synovial fluid, serum, and urine samples can be collected and compared to allow correlation of this marker in these different fluids. This may lead to translational benefit to human OA patients. The purpose of this report was to validate the use of the Urine Pre-Clinical CTX II assay for use with equine urine for future use with equine models of OA.
Methods: Urine Pre-Clinical Cartilaps® ELISAs (Nordic Bioscience Diagnostics) were used for this validation study according to manufacturer protocols. Internal quality control (QC) samples were prepared using the highest concentration standard provided by the manufacturer (99 ng/ml). To create QC samples, fresh urine was collected from 6 normal horses via catheterization. The samples were pooled together for further processing and analysis. Pooled samples were spiked with a known amount of standard to create samples with high, medium and low levels of CTX II. The QC samples were used to determine the precision, specificity, sensitivity, accuracy, linearity of dilution, and stability of this assay with equine urine. To ensure that the assay could detect different biological activity, 3 previously stored urine samples from normal and OA horses were analyzed.
Results: Reproducibility of the standard curve was evaluated (n=6 plates) by computing mean optical density (OD) and percent coefficient of variation (% CV) at each standard concentration. The overall mean inter-assay CV of the standard OD values was 6.5% (range 1.6-24.4%). Samples exhibited acceptable intra-assay and inter-assay precision over 3 plates with an overall mean CV of 4.3% (range; 1.2-12.3%) and 7.2% (range; 4.7-9.7%), respectively. Parallelism and linearity of dilution of equine serum sample dilutions (1:2, 1:4, 1:8, and 1:10) were demonstrated (Figure 1). Lowest detection limit of the assay was determined to be 1.38 ng/ml. Percent recovery was 90% for high, 90% for medium, and 95% for low QC samples. There was no loss in the stability of the CTX II epitope out to 4 freeze/thaw cycles. Differences in biological activity were identified when examining 3 samples from normal (3.23 $\pm$ 0.38 ng/ml) and OA (2.25 $\pm$ 0.37 ng/ml) horses (P=0.03) (Figure 2).