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Plasma Concentrations of a Type II Collagen-derived Peptide and Its Nitrated Form in Growing Ardenner Sound Horses and in Horses Suffering from Juvenile Digital Degenerative Osteoarthropathy

J.-Ph. Lejeune[1], D. Serteyn[1,2,3], M. Gangl[2], N. Schneider[1], G. Deby-Dupont[3], M. Deberg[4] and Y. Henrotin[3,4]

[1]*Centre Européen du Cheval, Mont-le-Soie, Vielsalm;* [2]*Department of Clinical Sciences, Large Animal Surgery, Faculty of Veterinary Medicine, University of Liège, Liège;*[3]*Center for Oxygen Research and Development, University of Liège, Liège;* [4]*Bone and Cartilage Research Unit, University of Liège, Institute of Pathology, Liège Belgium.*

ABSTRACT

Several breeds of draft horses suffer from degenerative digital osteoarthropathy, resulting in a reduced active lifespan. A group of 30 Ardenner horses was followed, in standardized conditions, from 15 to 28 months of age to detect the early manifestations of the disease. The severity of the disease was assessed according to a personal grading system including clinical and radiographic items. Coll 2-1, a peptide of the helical region of type II collagen, and its nitrated form (Coll 2-1 NO_2) were assayed in blood plasma collected at 452 ± 18 days, 504 ± 20 days, 558 ± 18 days, 613 ± 19 days, 675 ± 19 days, 752 ± 21 days and 852 ± 19 days of age. At the end of the follow-up period, 53.3% of Ardenner horses were affected by a degenerative digital osteoarthropathy. A significant effect ($p < 0.05$) of time, sex and pathology was observed for Coll 2-1 NO_2. Variations of Coll 2-1 were not significant except for the time effect. The elevation of Coll 2-1 NO_2 in the pathological group could indicate an inflammatory process during the growth of the affected horses, as nitration of tyrosine is mediated through reactive oxygen/nitrogen species and/or myeloperoxidase activity. Coll 2-1 NO_2 appears to be an interesting early marker of cartilage degradation and oxidation in degenerative osteoarthropathy.

Keywords: Ardenner horses, type II collagen, degenerative digital osteoarthropathy

Abbreviations: AAEP, American Association of Equine Practitioners; BSA, bovine serum albumin; Coll 2-1 NO_2, nitrate Coll 2-1; COMP, cartilage oligomeric matrix protein; CPU, carboxypeptide type II; ECM, extracellular matrix; EDTA, ethylenediaminetetraacetic acid; KS, keratan sulphate; LSM, least-squares mean; MMP, matrix metalloproteinase; MPO, myeloperoxidase; OA, osteoarthritis; PBS, phosphate-buffered saline; ROS, reactive oxygen species

INTRODUCTION

Joint diseases are a major cause of lameness in horses and have considerable negative economic consequences (Robert *et al.*, 1995; Caron, 2003). Among these joint diseases, the interphalangeal degenerative osteoarthropathy is probably one of the most frequent observed in adult draught horses. Further, this disease is commonly found in young draught horses, suggesting a juvenile form of this pathology (Lejeune *et al.*, 2002). Juvenile digital degenerative osteoarthropathy is characterized by forelimb lameness and by radiographic criteria including bone sclerosis, osteophytes, entheseophytes and remodelling of articular margins (May, 1996; Dyson, 2003; Ruggles, 2003). The causative factors of juvenile digital degenerative osteoarthropathy have not yet been identified and the pathogenic pathways remain speculative. Biomarkers of cartilage degradation are potentially useful for elucidating the dynamic changes in joint tissue and for early prognosis. Indirect biomarkers include proteolytic enzymes and their inhibitors, growth factors and pro-inflammatory cytokines. Matrix metalloproteinases (MMPs) play an important role in remodelling and degradation of the proteoglycans and the collagen component of the extracellular matrix of articular cartilage (McIlwraith, 2005). In juvenile horses, stromelysin or matrix metalloproteinase-3 (MMP-3) activity in synovial fluid was demonstrated to be twofold to threefold higher than in synovial fluid of adult horses (Brama *et al.*, 2000), and higher in 5-month-old than in 11-month-old foals. In adult horses, MMP activity of synovial fluid was independent of age but was nearly twofold higher in osteoarthritis (OA) than in normal joints (Brama *et al.*, 1998). In adult horses, a significant increase of gelatinase (MMP-9) activity was found in synovial fluids of osteoarthritic joints with severe cartilage lesions (Jouglin *et al.*, 2000). Direct biomarkers of cartilage metabolism include markers of the anabolic process such as carboxypeptide type II (CPII), chondroitin sulphate and markers of the catabolic process such as glycosaminoglycans, keratan sulphate (KS), cartilage oligomeric matrix protein (COMP) and type II collagen

fragments. Previous studies have associated cartilage alterations and increased concentrations of keratan sulphate in plasma of horses with osteochondral (chip) fractures, inflammatory arthritis (synovitis), infectious arthritis or osteochondrosis (Todhunter *et al.,* 1997). More recent papers have concluded that this marker had very little actual or potential value in the horse (Frisbie *et al.,* 1999; Mcllwraith, 2005). Cartilage lesions were demonstrated by significantly higher degraded COMP levels in synovial fluid of OA horses (Arai *et al.,* 2005). Collagenase-generated neoepitopes of type II collagen fragments, types I and II collagen fragments (COL2-3/4C(short)), and cross-linked telopeptide fragments of type I collagen were also reported as positive indicators of osteochondrosis severity at 5 months of age. In foals with lesions at 11 months of age, severity of osteochondrosis was correlated negatively with COL2-3/4C(short) and positively with CPII (Billinghurst *et al.,* 2004).

Recently, Henrotin and colleagues have developed two original immunological methods for measuring in the plasma concentration of a peptide derived from the triple helical region of type II collagen either in its native form (^{108}HRGYPGLDG^{116}, Coll2-1) or in its nitrated form (^{108}HRGY(NO_2)PGLDG^{116}, Coll 2-1NO_2) (Henrotin *et al.,* 2004; Deberg *et al.,* 2005). Peptide or protein nitration can result from the reaction of aromatic amino acid residues with peroxynitrite anion ($ONOO^-$), a strong oxidant formed by the reaction of nitric oxide (·NO) with superoxide anion ($O2^-$) (Beckman *et al.,* 1994) or from the activity of the oxidant enzyme myeloperoxidase (Kettle *et al.,* 1997; Burner *et al.,* 2000; Baldus *et al.,* 2002). Tyrosine is an aromatic amino acid residue that is particularly sensitive to nitration (Van der Vliet *et al.,* 1994). The measurement of the nitrated form of Coll 2-1 can thus be used as an indicator of oxidant activity in articular pathologies. Indeed an increased production of reactive oxygen species (ROS) in the diseased equine joint (traumatic injury with chip fragmentation and osteochondrosis dissecans) has been indirectly evidenced by measuring protein carbonyl content in synovial fluids, indicating that ROS were implicated in the pathogenesis of joint disease in horses (Dimock *et al.,* 2000). Human articular chondrocytes also were demonstrated to produce nitric oxide in response to cytokines (Stadler *et al.,* 1991 ; Henrotin *et al.,* 1998). Altogether, these data indicate that ·NO or derived reactive oxygen species are involved in cartilage degradation and suggest that cartilage matrix components can be nitrated *in situ* or in synovial fluid, and thereafter released into the blood (Kaur and Halliwell, 1994).

In humans, Coll2-1 and Coll2-1 NO_2 have been found to be increased in sera of patients with knee osteoarthritis (OA) or rheumatoid arthritis, and Coll2-1 NO_2, but not Coll2-1 in serum was correlated with C-reactive protein levels, suggesting that Coll2-1 NO_2 could be a marker of joint inflammation (Henrotin *et αl.,* 2004; Deberg *et al.,* 2005).

This work was designed to measure Coll2-l and Coll2-l NO_2 in the plasma of growing Ardenner horses and to determine the influence of sex and juvenile degenerative osteoarthropathy on the levels of these markers.

MATERIAL AND METHOD

Animals

The study was initiated with 32 horses (14 females and 18 males). All the animals registered in the Belgian stud book and were gathered from weaning at the centre Européen du cheval in Mont-le-Soie (Belgium). This group of 32 represented 9.3% of the births in Belgium in 2000. The animals were born between 17 April and 22 August, mainly in May and June. The average inbreeding coefficient was calculated using the method of Golden and colleagues (1991) on the basis of nearly complete 4-generation pedigrees and was 0.011 ± 0.027. Two males were lost due to injury, leaving 30 horses that were included in the trial.

Standardized conditions

The horses were divided into three groups (two groups of 9 males and one group of 14 females) and raised separately. They were maintained in pastures in all seasons and during winter were fed on a 12% protein mixture and hay of good quality *ad libitum.* The mixture was distributed at the rate of 1 kg/100 kg body weight divided into two meals. During summer, pasture was adequate to sustain feeding. Transitional periods were respected between seasons. Hoof trimming according to the rules that maintain the biomechanical balance of the foot (Caudron, 1997) was performed regularly.

Pathological classification

The radiographic and clinical data were available for 30 out of 32 horses as two (males) were injured and excluded from the research. Juvenile digital degenerative osteoarthropathy was diagnosed and graded according

to a personal scale combining radiographic and clinical criteria. Latero-medial and dorso-palmar radiographs of the weight-bearing digit were performed at the age of 852 ±19 days. The clinical evaluations were performed at 675 ± 19 days, 752 ± 21 days and 852 ± 19 days of age. A global assessment of the clinical status of the horse was established after the three clinical evaluations. A horse was considered lame when lameness was observed, at least once, consistently under all circumstances (without performing functional tests). This corresponds to a grade 3/5 of the grading system of the American Association of Equine Practitioners (AAEP, 1999): lameness was consistently observable at trot under all circumstances (such as weight carrying, circling, inclines and hard surfaces). A horse was also considered lame when the functional tests (lower limb flexion tests and wedge tests) revealed lameness on at least two of the three clinical evaluations. This situation corresponded to a grade 1/5 or 2/5 of the grading system of the AAEP: respectively, lameness difficult to observe and not consistently apparent regardless of circumstances, or lameness difficult to observe at a walk or trotting a straight line but consistently apparent under certain circumstances. Regional anaesthesia (palmar digital analgesia) was performed on the horses with severe lesions of the digit that were clinically lame. A positive response was observed in all the horses, confirming the localization of their lameness in the foot. The absence of lame horses, free of radiographic lesions, as well as the absence of sound horses with at least moderate radiographic lesions, made it possible to divide horses into two categories according to the radiographic data concerning phalanges changes and to the clinical results. The horses with slight to severe radiographic lesions and lameness were classified as 'pathological horses'. This group represented 53.3% of the horse cohort. The horses free of radiographic lesions and clinically sound or horses with slight radiographic lesions and clinically sound were considered as 'healthy horses'. This group included 46.7% of the cohort.

Blood samples

Venous blood samples were obtained from the jugular vein and collected into tubes containing EDTA (1 mg/ml). Blood was centrifuged at 3000g for 10 min and the plasma was aliquoted and frozen at -20°C until assayed. The blood samples were taken from the 30 horses at different periods of evaluation corresponding to the ages of 452 ± 18 days, 504 ± 20 days, 558 ± 18 days, 613 ± 19 days, 675 ± 19 days, 752 ± 21 days, and 852 ± 19 days, corresponding to the periods August-September 2001, October-November 2001, November-December 2001, January-February 2002, March-April 2002, May-July 2002 and September-October 2002, respectively.

Coll 2-1 and Coll 2-1 NO_2 immunoassays

These assays are competitive immunological methods described in detail by Deberg and colleagues (2005). Briefly, microplates were coated with 200 μl of streptavidin 0.5 mg/ml for at least 48 h. After washing (washing buffer: Tris 25 mmol/L, NaCl 50mmol/L, Tween 20 0.2% pH 7.3 (Sigma-Aldrich, Bornem, Belgium)), microtiter plates were subsequently blocked with 400 μl/well of blocking buffer (KH_2PO_4 1.5 mmol/L, Na_2HPO_4 8 mmol/L, KCl 2 mmol/L, NaCl 138 mmol/L, BSA 0.5%, monohydrate lactose 5.3g/100ml pH 7.2 (Sigma-Aldrich)) overnight at 4°C. Coll 2-1 and Coll 2-1 NO_2 were conjugated to biotin according to the method described by Rosenquist and colleagues (1998). After washing, 100 μl of the biotinylated peptides, either Coll 2-1 at 2.5 ng/ml or Coll 2-1NO_2 at 1.25 ng/ml, were added to each well of streptavidin-coated plates and incubated for 2 h at room temperature. Fifty μl of calibrators (synthetic peptide) or unknown samples, diluted 8-fold in dilution buffer (10mmol/L PBS, 138mmol/L NaCl, 0.7% BSA, 0.1% Tween 20 pH 7.0 (Sigma-Aldrich) for the Coll 2-1 immunoassay; 50mmol/L Tris, 138mmol/L NaCl, 0.7% BSA, 0.1% Tween 20 pH 8.0 (Sigma-Aldrich) for the Coll 2-1 NO_2 immunoassay), were applied to the wells, followed by either 100 μl of anti Coll 2-1 antibody, diluted 1/40000, or 100 µl of anti-Coil 2-1 NO_2 antibody, diluted 1/500000, and incubated for 1 h at room temperature. The dilutions of the antisera and of the secondary antibody were done in 10mmol/L PBS, 138mmol/L NaCl, 0.2% BSA, 0.1% Tween 20 pH 7.0 (Sigma-Aldrich) for the Coll 2-1 immunoassay, and in 50 mmol/L Tris, 138 mmol/L NaCl, 0.2% BSA, 0.1% Tween 20 pH 8.0 (Sigma-Aldrich) for the Coll 2-1 NO_2 immunoassay. After washing, 100 μl of peroxidase-conjugated goat antibodies to rabbit IgG (Biosource Europe SA, Nivelles, Belgium), diluted 1/5 000, were incubated 1 h at room temperature. After washing, 100 µl of freshly prepared enzyme substrate (Biosource) was added to each well. After 15 min, the reaction was stopped with 100 μl of 4 mol/L H_3PO_4. The coloration was read with an iEMS Reader MF microplate reader (Labsystem , Helsinki, Finland) at 450 nm, corrected for absorbance at 650 nm. Coll 2-1 immunoassay did not recognize native type I and II collagens, denaturated type I and II collagens, and BSA. Coll 2-1 NO_2 immunoassay did not recognize nitrated type I and II collagen, nitrated BSA and the 3-nitro-L-tyrosine residue, but a low cross-reactivity of 0.02% with Coll 2-1 was observed. The limits of detection were 17 nmol/L and 50 pmol/L for Coll 2-1 and Coll 2-1 NO_2 immunoassays, respectively. For both immunoassays, the coefficients of variation were below 10%. To avoid a matrix effect, the plasma samples should be diluted at least 8-fold. The spiking recovery ranged from 101.8% to 106.5% for Coll 2-1 and from 106.2% to 132.2% for Coll 2-1 NO_2 immunoassays, respectively.

Statistical analyses

The data followed a global linear model. The mixed procedure of SAS was used. The global effects of time, pathology and sex were analysed (test of fixed effects). Variations of the least-squares means (LSM) were considered signicantly different when $p < 0.05$. Interactions between sex, pathology and time were tested. Comparison of the LSM of the plasma concentrations of Coll 2-1 and Coll 2-1 NO_2 between males and females and between pathological and healthy groups at each period of sampling were evaluated by the least significant difference (LSD) test of Fisher (Post-Hoc tests, Statistica, Version 6.1). A decrease or an increase was considered significant if $p < 0.05$.

RESULTS

The groups of pathological and healthy horses represented 53.3% and 46.7% of respectively, the horses. The group of pathological horses included 7 females and 9 males, while the group of healthy horses included 6 females and 8 males. We observed a significant global effect of time for both markers, and a significant difference for Coll2-1 NO_2 between healthy horses and pathological horses and between females and males. LSM of the plasma concentrations are reported in Table I. Interactions between sex, time and pathology were not significant for either marker except for interaction between time and sex for Coll 2-1 NO_2.

Table I : *Effects of sex and pathology (osteoarthropathy) on the plasma markers of cartilage metabolism, Coll 2-1 and Coll 2-1 NO_2, in a group of 30 Ardenner horses using the entire data from 452 ±18 days to 852 ±19 days of age. The values (nmol/L) are the least-squares means calculated by the mixed procedure of SAS.*

	Sex effect			Pathology effect		
Marker	**Females**	**Males**	***p* value**	**Healthy**	**Pathological**	***p* value**
Coll 2-1	875 ± 47	927 ± 43	NS	918 ± 46	884 ± 44	NS
Coll 2-1 NO_2	2.91 ± 0.12	2.15 ± 0.11	<0.05	2.31 ± 0.12	2.76 ± 0.12	<0.05

NS, not significant

The evolution of both markers is illustrated in Figure 1. Coll 2-1 and Coll 2-1 NO_2 showed a similar profile during the growing phase. They decreased progressively between day 558 and days 675, reached a minimum at 675 ± 19 days, corresponding to the spring of the year 2002, and thereafter increased until 852 ± 19 days. The minimum mean values observed at 675 ± 19 days were significantly different from the values at 452 ±18,504 ± 20,558 ± 18 and 852 ± 19 days ($p < 0.01$; $\rho < 0.01$; $\rho < 0.05$; $p < 0.001$) for Coll 2-1 and from the values at 452 ± 18 and 852 ± 19 days ($p < 0.05$; $p < 0.001$) for Coll 2-1 NO_2. At 852 ± 19 days, the plasma concentrations of both markers were significantly higher than at 558 ± 18, 613 ± 19, 675 ± 19 and 752 ± 21 days ($p < 0.05$; $p < 0.01$; $p < 0.001$; $p < 0.01$) for Coll 2-1 and at 613 ± 19, 675 ± 19 and 752 ± 21 days ($p < 0.01$; $p < 0.001$, $p < 0.05$) for Coll 2-1 NO_2. The minimum levels of Coll 2-1 and Coll 2-1 NO_2 in plasma were 754.23 ± 47 nmol/L and 2.23 ± 0.13 nmol/L, respectively at 675 ± 19 days, and the maximum values were 1046.78 ± 47 nmol/L and 2.86 ± 0.13 nmol/L at 852 ± 19 days.

The plasma concentrations of Coll2-1 were similar in males and females (data not shown), whereas Coll2-l NO_2 plasmatic levels were significantly higher in females than in males (Figure 2) at 452 ± 18, 504 ± 20, 558 ± 18, 675 ± 19,752 ± 21 and 852 ± 19 days ($p < 0.05$; $p < 0.001$; $p < 0.05$; $p < 0.05$; $p < 0.001$; $p < 0.01$).

The plasma concentrations of Coll2-l NO_2 were significantly higher in the pathological group than in the healthy group (Table I). Variations during growth are illustrated in Figure 3: the mean value of pathological group was significantly higher ($p < 0.05$) at 504 ± 20,558 ± 18 and 852 ± 19 days of age.

DISCUSSION

We used two competitive immunoassays specific for measuring a breakdown product of type II collagen (Coll 2-1) and its nitrated form (Coll 2-1 NO_2) (Henrotin *et al.*, 2004; Deberg *et al.*, 2005). The novel aspect of the study lies in the fact that these markers reflect both collagen breakdown and oxidation phenomena that could have occurred in the matrix.

During growth of the horses Coll 2-1 and Coll 2-1 NO_2 had parallel evolution profiles, with a minimum in the plasma concentrations in spring, when grass was growing and when grass formed the major part of the horses' diet.

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Figure 1 : *Evolution of the least-squares means (LSM) of the plasma concentrations (nmol/L) of Coll 2-1 (dotted curve) and Coll 2-1 NO_2 (solid curve) in a group of 30 Ardenner horses from 452 ±18 to 852 ±19 days of age. The x-axis represents the average age (in days) at blood sampling: 452 ±18,504 ±20,558 ±18,613 ±19 ,675 ± 19,752 ±21 and 852 ±19 days. These ages correspon d, respectively, to the approximate dates August-September 2001, October-November 2001, November-December 2001, January-February 2002, March-April 2002, May-July 2002 and September-October 2002. The lowest values are observed at 675 ±19 days of age, corresponding to the spring 2002. The error bars represent the standard deviation.*

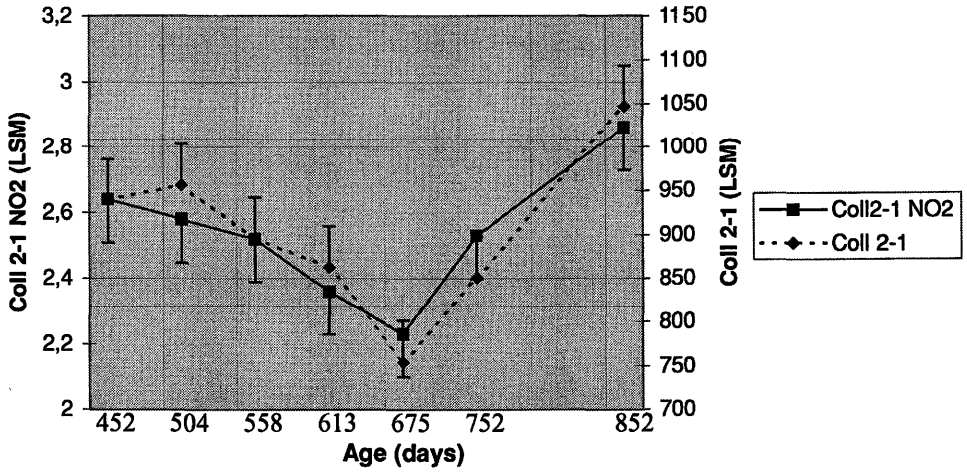
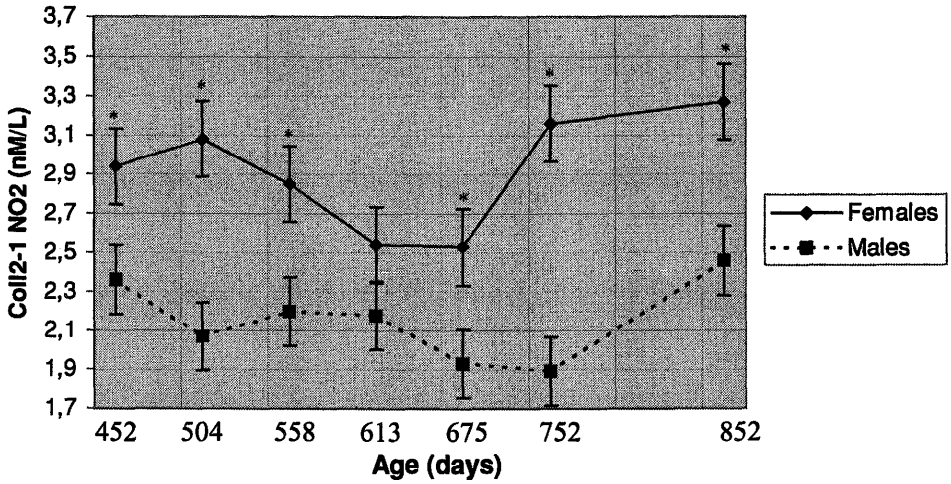


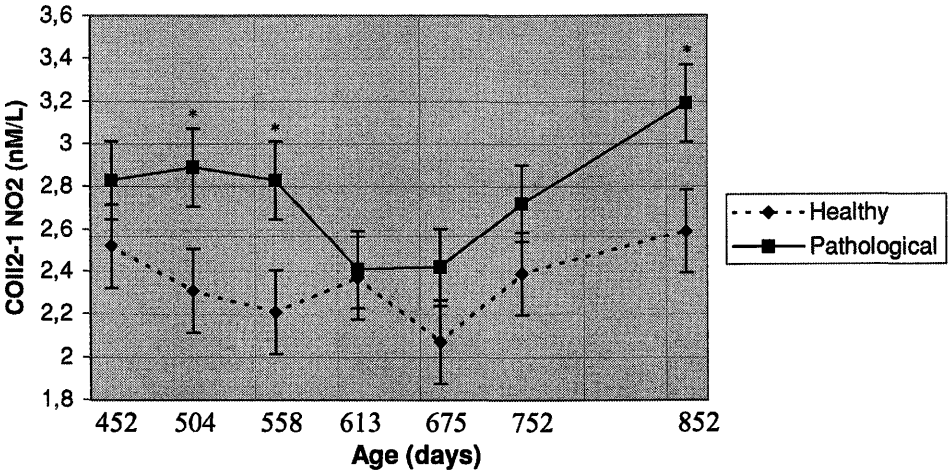
Figure 2 : *Evolution of the least-squares means of the plasma concentrations (nmol/L) of Coll 2-1 NO_2 in females (solid curve) and males (dotted curve) in a group of 30 Ardenner horses from 452 ±18 to 852 ± 19 days of age. The x-axis represents the average age (in days) at blood sampling: 452 ±18,504 ±20,558 ±18, 613 ± 19,675 ±19,752 ±21 and 852 ±19 days. These ages correspond, respectively, to the approximate dates August-September 2001, October-November 2001, November-December 2001, January-February 2002, March-April 2002, May-July 2002 and September-October 2002. The error bars represent the standard deviation. Asterisks show time points at which the groups were significantly different. We observed a significant global effect between females and males.*



The analysis of the least-squares means of Coll 2-1 did not show any significant difference between the sexes, corroborating observation in humans (Deberg *et al.*, 2005). However, Coll 2-1 plasma concentrations were not significantly different between healthy and pathological horses, in disagreement with the results obtained in humans, in whom Coll 2-1 concentrations were significantly increased in OA patients, indicating an increase in type II collagen degradation. This difference of Coll 2-1 between pathological horses and OA patients might partially be explained by the early stage of the equine pathology in our study group. Indeed, the radiographic lesions that we observed in this study concerned mostly new bone formation, entheseophytes on the phalanges, at this stage of the disease. It is likely that the major cartilage lesions of the interphalangeal joints appear later.

For Coll 2-1 NO_2, we observed that plasma concentrations were higher in females than in males, corroborating the results already obtained in humans. A possible explanation is that oestrogens increase protein nitration through an increase of $^{\cdot}NO$ production. Estradiol has been demonstrated to act as a pro-oxidant, promoting neutrophil degranulation (Chiang *et al.*, 2004). Oestrogens could also have a potential effect on chondrocytes by stimulating the expression of the inducible nitric oxide synthase (iNOS), as has been demonstrated in endothelial cells (Cho *et al.*, 1999; Mershon *et al.*, 2002; Liang *et al.*, 2003).

Figure 3 : *Evolution of the least-squares means of the plasma concentrations (nmol/L) of Coll 2-1 NO_2 in pathological (solid curve) and healthy horses (dotted curve) of a group of 30 Ardenner horses from 452 ± 18 to 852 ± 19 days of age. The x-axis represents the average age (in days) at blood sampling: 452 ± 18,504 ± 20, 558 ± 18,613 ± 19,675 ± 19,752 ± 21 and 852 ± 19 days. These ages correspond, respectively, to the approximate dates August-September 2001, October-November 2001, November-December 2001, January-February 2002, March-April 2002, May-July 2002 and September-October 2002. The error bars represent the standard deviation.*



The most original observation of this work was the significant increase of the Coll 2-1 NO_2 plasma concentrations in horses developing a progressive digital osteoarthropathy, compared to the healthy horses. The global effect of the pathology was significant on this marker, indicating that the increase of Coll2-l NO_2 could be of predictive value for juvenile digital degenerative osteoarthropathy. When evaluating each age separately, the difference between the two groups remained stable over the experimental period but was significant only at three of seven ages. More studies are needed to confirm and define a predictive value of this new marker. The presence of a peptide derived from the extracellular matrix type II collagen and nitrated on its tyrosyl residue undoubtedly indicated the presence of oxidant activity, but did not indicate where the oxidant activity occurred. Two mechanisms can be invoked to explain the *in vivo* tyrosine nitration. The first is the reaction of tyrosine oxidation by peroxynitrite ($ONOO^-$), an unstable oxidant formed during the near-diffusion-limited reaction of superoxide anion ($O_2^{\cdot -}$) with nitric oxide ($^{\cdot}NO$) (Beckman *et al.*, 1994; Baldus *et al.*, 2002; Brennan *et al.*, 2002). In pathological conditions such as arthritis and anoxia-reoxygenation, superoxide anion and nitric oxide are produced by chondrocyte, but also by inflammatory cells found in synovial fluid and by synovium (Henrotin *et al.*, 1993, 1998, 2003; Stichtenoth and Frölich, 1998). Additional examinations such as synovial fluid analysis could be useful in investigating the presence of an articular inflammatory process. Recently, a second pathway for tyrosine nitration has been suggested. This involves the neutrophil enzyme myeloperoxidase (MPO) (Kettle *et al.*, 1997; Burner *et al.*, 2000; Baldus *et al.*, 2002; Brennan *et al.*, 2002; Gaut *et al.*, 2002). MPO is released when neutrophils are stimulated during inflammatory reaction. Increased MPO activity was observed in antigen-induced arthritis in guinea pigs and was attributed to neutrophils attracted to synovial tissue (Kuwabara *et al.*, 2002). Kubala and collaborators (2004a,b) reported that MPO was sequestered within extracellular matrix (ECM) proteins in a glycosaminoglycan-dependent manner and that the enzymatic activity of MPO was increased when associated with ECM proteins. In our study, the nitrated peptide was measured in the plasma. Therefore, the tyrosyl residue could have been nitrated either in the articulation (in the cartilage matrix, in the synovial fluid, or in the synovial membrane) or in the plasma. To explore where and through which pathway the tyrosyl residue was nitrated, measurements of Coll 2-1 and Coll 2-1 NO_2 levels in synovial fluid would be interesting, as well as concomitant measurement of MPO level in plasma and synovial fluid.

In conclusion, the increase of Coll 2-1 NO_2 plasma concentrations clearly demonstrated an increase of collagen fragment nitration, and therefore implicated an inflammatory phenomenon in the pathophysiology of juvenile

degenerative digital osteoarthropathy in draught horses. Further studies are needed to elucidate the site and origin of the nitration of the collagen breakdown marker. As Coll 2-1 NO_2 reflects simultaneously both collagen breakdown and inflammation, the measurement of its plasma or synovial concentration can be considered a new and valuable tool for studying the pathophysiology of various equine joint diseases.

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