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Invited review

How exercise influences equine joint homeostasis

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ABSTRACT

The maintenance of joint homeostasis is integral to joint health. Knowledge of the influence of exercise on joint homeostasis is not only relevant for determining sustainable levels of equine athletic training, but also for the study of early development of osteoarthritis or cartilage repair in animal models. This review provides an overview of findings derived from in vivo studies and postmortem analyses investigating exercise effects on various joint tissue components in the horse, supplemented where appropriate with data from small animal models. The concept of joint homeostasis and possible methods to quantify this are also discussed, with special attention to the potential benefits and pitfalls of biomarker analysis in synovial fluid. The main conclusion is that biomechanical loading in the form of deliberate exercise has a major influence on the delicate homeostatic balance within the tissues constituting the diarthrodial joint and on their interactions, which is crucial for proper and durable joint function. The amount and intensity of exercise can have a lasting effect on tissue characteristics in juvenile animals, but affects joint homeostasis in mature animals and can affect the delicate balance between physiologic adaptation and development of pathology. Biomarkers in synovial fluid can be helpful in assessing joint homeostasis, but their use and interpretation require caution and are often far from straightforward.

Introduction

Although the ability of living beings to maintain stability of their internal milieu were proposed by Claude Bernard in the 19th century (Bernard, 1878), Walter B. Cannon was the first who named this concept 'homeostasis' (Cannon, 1926). Internal disturbances caused by changes in the environment, affecting the system indirectly or directly, are kept within narrow limits by automatic adjustments to preserve normal tissue function and development. The concept applies to the body in general, but also to its constituent elements (Cannon, 1929). In a functional, healthy joint, a dynamic equilibrium between cellular processes within and between articular tissues is maintained. Disturbances in joint homeostasis that cannot be kept within physiological limits cause an imbalance between catabolic and anabolic processes and can lead to the onset and progression of joint disorders such as osteoarthritis (OA; Goldring and Marcu, 2009; de Grauw, 2011; Chu and Andriacchi, 2015).

The horse has always been kept primarily for its athletic performances, both in historical roles in warfare, transport and agriculture, and nowadays in its role as a sport and leisure animal. This emphasis on physical exercise means that high demands are placed upon the equine musculoskeletal system. In particular, the appendicular joints are subject to substantial compressive and shear forces and consequently, joint damage leading to impaired mobility and lameness is an important concern in the horse industry (Penell et al., 2005; Dyson et al., 2008). It is clear from the published literature that exercise, both in terms of intensity and duration, plays an important role in joint development and function (Brama et al., 2002a; Vigre et al., 2002; Steel et al., 2006).

At the two extremes, both joint immobilisation and the sudden application of substantial mechanical force can lead to degenerative changes of the articular cartilage (Vanwanseele et al., 2002; Anderson et al., 2011). Although physical exercise is the most recommended non-pharmacological intervention for human OA patients (McAlindon et al., 2014), its efficacy depends on various factors and it might not always be beneficial (Regnaux et al., 2015; Liu et al., 2016). In the horse, regular canter exercise has been reported to be generally beneficial for joint health, whereas prolonged high-speed training could be a risk factor for metacarpo- and metatarsophalangeal joint injury (Reed et al., 2013). This indicates that exercise influences joint homeostasis and can up- or downregulate anabolic or catabolic processes. There appears to be a window in which exercise has beneficial effects on the joint, while outside this window, joint homeostasis is disturbed to such a degree that compensatory mechanisms cannot cope (Hallett and Andrish, 1994).

Appreciation of the effects of exercise in physiological situations is not only relevant for establishing sustainable levels of athletic training in the (young) performance horse. It is also necessary to understand the influence of exercise on the onset and progression of joint pathology, as well as its influence on the capabilities of articular tissues to heal or regenerate (Van den Hoogen et al., 1998;

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Saris et al., 2003). In addition, injured tissues and healthy tissues might respond differently to mechanical loading (Khan and Scott, 2009). These are important considerations when studying early OA development or effects of (tissue-engineered) cartilage repair strategies in experimental animal models. This review concentrates on findings derived from in vivo studies and postmortem analyses investigating the influence of exercise on various joint components of the healthy equine joint, supplemented where appropriate with data from small animal models. Special attention is given to synovial fluid (SF) biomarker levels as surrogate outcome measures.

Defining and assessing 'joint homeostasis'

The diarthrodial joint is a complex organ consisting of several key components, including the subchondral bone, the articular cartilage, the synovial membrane lining the inner layer of the fibrous joint capsule and, in some joints, intra-articular structures such as ligaments and menisci. These elements closely interact directly and/or indirectly via the SF that fills up the joint cavity (Hui et al., 2012). The SF is an ultrafiltrate of plasma to which various components are added by articular tissues. Apart from its function as an important communication medium between the various tissues, the SF serves as a joint lubricant and it helps redistributing forces during loading and unloading. Furthermore, it fulfils an important role in the nourishment of the articular cartilage layer which is avascular in nature and thus depends mainly on SF for its nutrition and removal of waste products (Levick, 1995).

Joint homeostasis can be defined as the dynamic balance of catabolic and anabolic processes within and between all these joint components, which is required to maintain tissue integrity and biomechanical functionality through physiological cellular processes (de Grauw, 2011). Given the close interaction, the concomitant cross-talk and reciprocal feedback mechanisms between the various joint components, joint homeostasis should be considered a variable or dynamic equilibrium rather than a static state (de Grauw, 2011). Breaking down 'joint homeostasis' into numerous homeostatic processes and assuming that derangement in any of these might cause a disruption of homeostatic balance in general not only avoids oversimplification of a highly complex biological system, but also allows the assessment of single joint components or specific aspects of the joint tissue status; combining this information at a later stage can then help to obtain a comprehensive picture of joint homeostasis and its derangement (de Grauw, 2011; Hui et al., 2012).

Joint components can be isolated and studied in vitro. This can give important insights in molecular events responsible for beneficial or detrimental effects of specific interventions at the tissue level (Goldring and Marcu, 2009). However, in vitro studies might not reflect the complexity of the situation in vivo, since the interactions of each component with its natural environment, the large range of stress spectra in biomechanical loading, and individual differences, play important roles. For the clinical evaluation of joint health, diagnostic imaging and physical examination are useful parameters that can provide information about tissue structure and function. However, they reflect the structural or functional consequences of disturbed homeostasis rather than providing real-time information on the molecular processes inside the joint. Since SF undergoes continuous turnover and is in direct contact with all relevant joint tissues except the subchondral bone, its composition provides approximately real-time information on joint homeostasis (van den Boom, 2004). Furthermore, SF is the only joint component that can be obtained relatively easily from a live animal without causing significant tissue destruction. Therefore, molecular biomarkers in synovial fluid have been studied extensively in relation to joint diseases (Lotz et al., 2014) where early recognition of disruption of homeostatic processes can have high diagnostic and prognostic value (Chu and Andriacchi, 2015).

Mechanisms of exercise effects

'Exercise' is a rather broad term, usually referring to regular physical activity (or physical/mechanical loading) that is performed to improve fitness and strength. The mechanisms by which exercise can influence joint homeostasis are complicated and closely related. For the purpose of this review, we distinguish between three main mechanisms: (1) the direct effect of mechanical impact on articular tissue integrity; (2) the indirect influence on joint tissue metabolism; and (3) the influence of exercise on joint circulation (Fig. 1).

Direct mechanical effects of exercise on articular tissue integrity

Maintenance of the integrity of articular tissues in general and the articular cartilage extracellular matrix in particular is imperative to preserve the properties required to meet the functional demands placed upon the joint. Structural damage to the articular cartilage could in itself affect the force distribution in the joint (Schett et al., 2001), meaning that the limit of beneficial exercise might shift in the pathologic joint. The sudden application of substantial mechanical force, also referred to as 'impact', is a common cause of articular cartilage injury and often leads to post-traumatic OA (Buckwalter and Felson, 2015). Repeated joint loading results in an increase in subchondral bone thickness and density (Firth et al., 1999; Kawcak et al., 2000; Murray et al., 2007), subsequently leading to increased shear stresses at the base of the articular cartilage. This can cause deep horizontal splits which can progress to the articular surface, disrupting the integrity of the cartilage layer (Radin and Rose, 1986). In retired Thoroughbred racehorses of different ages, progressive subchondral micro-cracking was observed in the metacarpophalangeal joint in younger animals, escalating to severe subchondral bone collapse and concomitant lesion formation in the cartilage layer with increasing age and thus cumulative athletic workload (Turley et al., 2014).

Disruption of the integrity of articular tissues does not necessarily refer only to the existence of cracks or tears in cartilage or bone, as the accumulation of repair ('scar') tissue due to overuse could affect the balance between catabolic and anabolic processes (Hart and Scott, 2012). Inflammation of soft tissues (synovitis/capsulitis) due to trauma induced by (repeated) athletic activities can also contribute to degradative processes by the release of inflammatory mediators and cytokines into the SF (McIlwraith et al., 2012). The very limited repair capacity of the articular cartilage (Heinemeier et al., 2016) makes it particularly susceptible to initiation of a vicious cycle, in which micro-damage and subsequent inflammatory responses from the synovial membrane result in depression of chondrocyte synthetic activities and release of numerous catabolic mediators, causing further disturbance of joint homeostasis and articular cartilage breakdown (de Grauw, 2011; Fig. 2).

Influence of exercise on articular tissue metabolism

Mechanical loading modulates articular tissue metabolism by activating signal transduction pathways that translate mechanical stimulation into biochemical signals, altering cellular processes and leading to matrix remodelling (Khan and Scott, 2009). This 'adaptive response' can be detected in each of the joint components and has been studied extensively in equine articular cartilage. The juvenile equine joint in particular is an excellent example of how exercise influences tissue turnover, leading to changes in cartilage matrix composition.

Exercise-induced changes in juvenile articular cartilage matrix composition

Whereas mature cartilage is known to be a highly immutable tissue with minimal repair capacity due to the extremely long

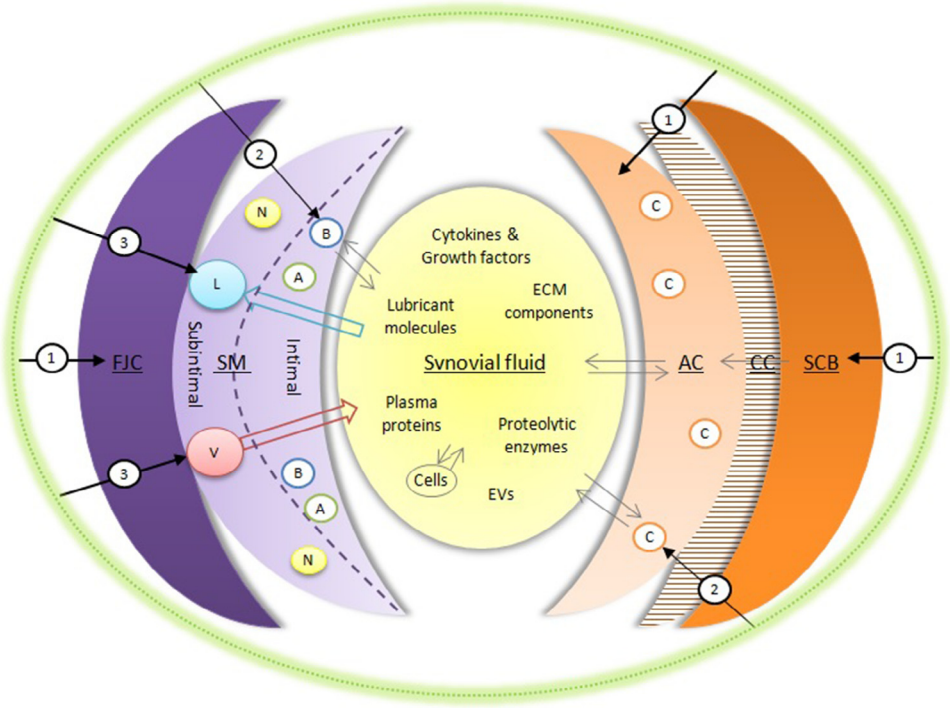

Fig. 1. Schematic representation of the mechanisms of exercise effects on joint homeostasis. SCB, subchondral bone; CC, calcified cartilage; AC, articular cartilage; SM, synovial membrane; FJC, fibrous joint capsule; L, lymph vessel; V, blood vessel; N, nerve fibre; A, type A/macrophage-like synoviocytes; B, type B/fibroblast-like synoviocytes; C, chondrocytes; EVs, extracellular vesicles. The green circle represents exercise that influences various joint components via (1) direct mechanical effects on SCB, AC and FJC, (2) synoviocytes and chondrocyte metabolism and (3) joint circulation. Grey open arrows represent interaction between different joint components.

turnover time of extracellular matrix (ECM) components and in particular of the collagen network (Heinemeier et al., 2016), the situation in juvenile animals is inherently different, as they are going through a process of active growth and development. The joint of a newborn foal displays a homogeneous distribution of proteoglycan and collagen content, including posttranslational modifications of collagen such as cross-links, across the articular cartilage. Over time, these tissue components reach mature levels and important structural changes occur (Little et al., 1997; Brama et al., 2002a). Over the past decades, several large-scale ex vivo studies in juvenile horses (from neonates to 18 months old) have provided important insights into the crucial role of exercise in articular cartilage maturation (Barneveld and van Weeren, 1999; Brama et al., 2002a; Rogers et al., 2008; van Weeren et al., 2008).

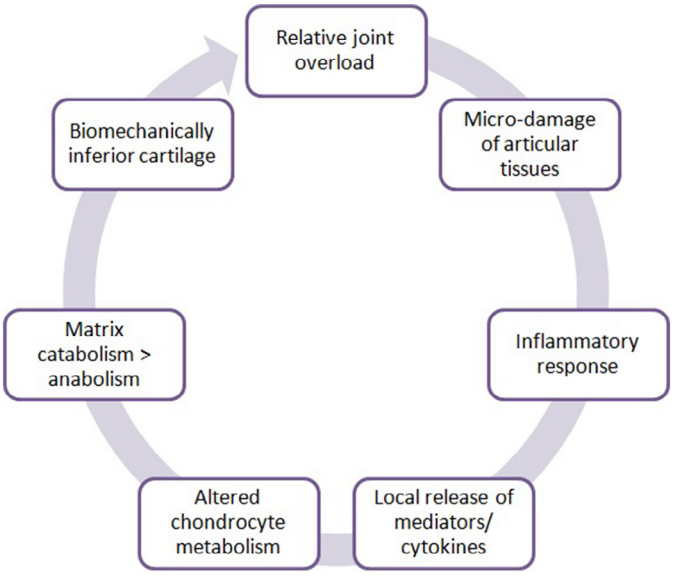

Fig. 2. Vicious cycle of articular cartilage breakdown (adapted from de Grauw, 2011).

Briefly, these studies led to the conclusion that withholding any form of exercise during the first 5 months of life results in the lack of formation of articular cartilage topographic heterogeneity, which could have serious consequences for future tissue resistance to injury (Brama et al., 2002a). However, an increased workload appeared to speed up the normal process of maturation of ECM components. By 18 months of age, articular cartilage from foals kept on pasture and subjected to additional trot and canter exercise showed a biochemical composition closer to that of mature animals when compared to pasture-kept foals without additional exercise (van Weeren et al., 2008). Advanced maturation of the ECM can be beneficial in horses that have to perform athletically at a very early age, because of the reinforcement of the cartilage ECM through increased matrix crosslinking. However, there might also be disadvantages. Although the advancement of the maturation process can be reversible in the case of glycosaminoglycans (GAGs), this is unlikely to be the case for collagen and collagen related structures. Therefore, it could be argued that foals subjected to additional training might reach a stage at which the collagen network no longer enables further cartilage remodelling. This could lead to a reduced capacity for repair, which is inherently disadvantageous (van Weeren et al., 2008). This suggests that even moderate exercise protocols can induce substantial changes in the biochemical composition of the articular cartilage if implemented at an early age. Therefore, the gap between the beneficial or detrimental effects of exercise on articular cartilage metabolism might be relatively narrow in young animals.

Exercise-induced changes in articular cartilage matrix composition in young adults

In 2-year-old horses, no overall effect on collagen content of the middle carpal articular cartilage has been observed after 19 weeks

of high-intensity treadmill exercise compared with daily walking exercise. However, exercise was shown to decrease collagen content at sites predisposed to clinical lesions (Murray et al., 2001a). In another study in 2-year-olds, it was concluded that strenuous exercise (i.e. race training) provoked significant alterations in the characteristics of the collagen network of the articular cartilage of the fetlock joint compared with a pasture-kept control group of the same age. These changes were suggestive of micro-damage and loosening of the collagen network (Brama et al., 2000).

For the most abundant non-collagenous protein present in articular cartilage, cartilage oligomeric matrix protein (COMP), exercise effects have been found at both intermittently loaded high weight-bearing (i.e. dorsal) joint areas and at more constantly loaded lower weight-bearing (i.e. palmar) areas of the cartilage in the middle carpal joint. In 2-year-old horses trained on a treadmill, COMP content was lower than in daily-walked control horses and it was mostly lower at dorsal sites than at palmar sites. In the control horses, this pattern was not observed (Murray et al., 2001b). In a study of 3-year-olds, COMP content was lower in animals that had been trained for 1.5 years and had raced regularly than in pasture-kept control horses. However, in contrast to the findings in 2-year-old treadmill trained horses, the pasture-kept controls had higher COMP content in dorsal areas than in palmar areas. These findings suggest that adequate dynamic loading promotes COMP synthesis, while excessive loading could have the opposite effect (Skiöldebrand et al., 2010).

For proteoglycans, anabolic effects of exercise have been reported. In 2-year-old treadmill exercised horses, GAG content was higher at heavily loaded sites of cartilage in the middle carpal joint than in cartilage from horses that underwent daily walking exercise (Murray et al., 2001a). In contrast, no significant difference in endogenous proteoglycan content was found after 6 weeks of exercise consistent with early race training compared with box-rested control horses (Palmer et al., 1995). However, in cartilage explants from the exercise group, the amount of newly synthesised proteoglycan was increased (Palmer et al., 1995). Enhanced proteoglycan synthesis and reduced proteoglycan breakdown has also been observed in equine cartilage explants cultured in SF obtained after moderate exercise compared with cultures in pre-exercise SF (Van den Hoogen et al., 1998). This supported the hypothesis that the effects of intensified loading on articular cartilage metabolism were not only a direct result of the transduction of mechanical stress on chondrocytes into biochemical signals, but that they might also be mediated by substances released into the synovial fluid, emphasising the complex interactions between the various articular components. Molecular markers in SF that could reflect changes in tissue metabolism are discussed separately in the next section.

It is worthwhile to briefly mention the consequences of the opposite of exercise, namely joint immobilisation, on articular cartilage metabolism. These have been studied extensively in adult dogs (Vanwanseele et al., 2002). Immobilisation of the stifle joint led to significant decreases in cartilage GAG content and synthesis. No changes in collagen content were found, but immobilisation reduced collagen cross-linkage (Haapala et al., 1999). To a certain extent, these changes were reversible during remobilisation, with remarkably better results when small movements during immobilisation were allowed (Behrens et al., 1989; Haapala et al., 1999).

Exercise induced changes in the metabolism of other articular tissues

Although under physiologic conditions, the subchondral bone is not in direct contact with the synovial cavity, it has an intimate physical association with the articular cartilage layer. Therefore, alterations of either tissue modulate the properties and function of the other, through biochemical and molecular crosstalk across their interface (Pan et al., 2009; Findlay and Kuliwaba, 2016). Crosstalk between bone and cartilage plays an important role in joint homeostasis and has been described extensively in relation to the pathogenesis of osteoarthritis (Findlay and Kuliwaba, 2016). For the mature equine carpal, metacarpophalangeal and tarsal joints, subchondral bone has been shown to undergo functional adaptation, influenced by exercise and by the specific site within the joint. Reported effects include regional changes in thickness and density, which tend to increase under the influence of loading (Firth et al., 1999; Kawcak et al., 2000; Murray et al., 2001c, 2007). Studies in foals have shown that, similar to articular cartilage, loading appears to play a key role in the development of site-related differences in the biochemical composition of subchondral bone (Brama et al., 2001). In foals withheld from exercise, bone calcium content and hydroxylysylpyridinoline and lysylpyridinoline crosslinks in subchondral bone of the proximal first phalanx were reduced compared with foals subjected to daily sprint training or foals kept at pasture 24 h per day. The fact that these differences were observed at the dorsal site, which sustained intermittent peak loading during exercise, and not at a more constantly loaded site, supports the role of biomechanical loading in subchondral bone metabolism, as dictated by Wolff's law (Wolff, 1870; Brama et al., 2002b).

Limited information is available on in vivo effects of physical loading on synovial membrane integrity and its metabolic activities, especially in the horse. In juvenile horses, effects of superimposed exercise on the synovial membrane have been examined histologically (Kawcak et al., 2010). Although detrimental effects such as cellular infiltration, hyperplasia, oedema, fibrosis or hyperemia were not reported in that study, these particular changes as well as markedly elevated expression of pro-inflammatory interleukin (IL)-15 expression have been observed in stifle joint synovial membrane of rabbits subjected to repetitive impulse loading or high-intensity treadmill training (Lukoschek et al., 1986; Walker et al., 1991; Wang et al., 2015). In rats, a long distance running protocol for 45 days led to increased synovial expression of matrix metalloproteinases (MMP)-1, -9 and -13 compared to cage-restrained animals (Shangguan et al., 2013). These findings suggest potential detrimental pro-inflammatory effects of supramaximal exercise on the synovial membrane.

Influence of exercise on joint circulation

Blood flow plays a major role in the efficiency of fluid exchange between synovial capillaries and the joint cavity and thus in the maintenance of joint homeostasis. It is influenced by motion (i.e. exercise), either directly or indirectly via alterations in the intra-articular pressure (IAP). Changes in joint angle cause a pulsatile capillary flow, and articular soft tissues, particularly the synovial membrane, have been shown to receive an increased blood flow in response to short-term treadmill exercise in the canine stifle and radiocarpal joint (Simkin et al., 1990). An alternating IAP in different joint compartments stimulates fluid exchange to the interstitium and lymph flow from the interstitium. These mechanisms promote the clearance and turnover of SF, maintaining the normally negative IAP (Fig. 3; Hardy et al., 1996; Bertone et al., 1998; da Gracca Macoris and Bertone, 2001).

Relative overload of the joint can lead to a disruption of this physiological mechanism through joint effusion, which could occur in response to high intensity exercise (Persson, 1971; Frisbie et al., 2008), or through reduced joint capsule compliance (fibrosis) due to traumatic synovitis/capsulitis. The net result is a pathological increase in IAP and eventually destruction of the capillary and neuronal network (Fig. 3; Eitner et al., 2013). This can have consequences for nociceptive and proprioceptive function of the joint, and loss of a normally innervated vascular bed could disturb circulation in the articular tissues. Moreover, increased IAP can cause great reduction

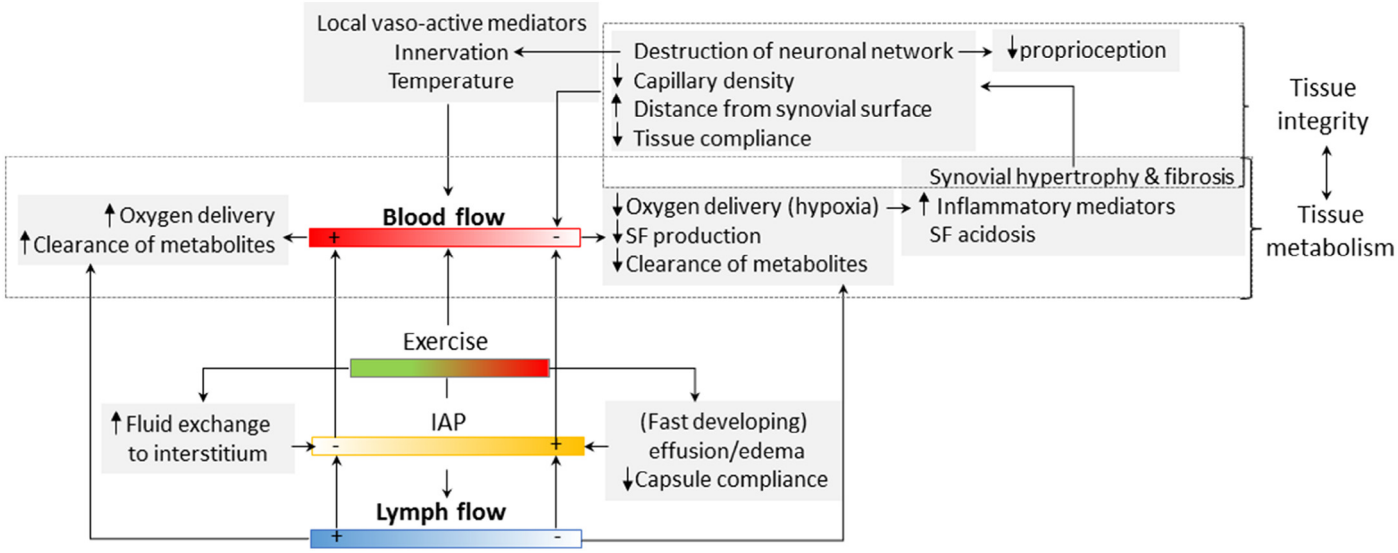

Fig. 3. Schematic representation of the effect of exercise on circulation in articular tissues and mechanisms involved with their relation to tissue integrity and tissue metabolism.

of synovial membrane blood flow (Hardy et al., 1996) and in effused joints, blood flow might be further compromised by modest elevations in IAP induced by exercise (James et al., 1990; da Gracca Macoris and Bertone, 2001). Decreased blood flow negatively affects both SF turnover and SF composition, as it leads to decreased filtration and consequently decreased SF production, as well as impaired oxygen delivery, resulting in local lactic acidosis (Hardy et al., 1996).

Synovial fluid biomarkers

As SF is a composite result of joint circulation and metabolic activity of the articular cartilage and synovial membrane, all effects of exercise on joint homeostasis described above change the molecular composition of the SF, and this might be reflected by changes in its biomarker profile. A biomarker can be defined as a 'characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' (Biomarkers Definitions Working Group, 2001). A practical approach classifies biomarkers in SF into direct and indirect markers (Sipe, 1995). Direct markers include cleavage fragments and synthesis (by-)products of articular tissue components that directly reflect anabolic and catabolic processes, for example markers related to collagen or aggrecan metabolism, lubricant molecules or COMP (Saxne and Heinegård, 1992). Indirect biomarkers are molecules that affect tissue metabolism, but are not generated during tissue synthesis or breakdown, e.g. enzymes, inflammatory mediators, cytokines and growth factors. Direct and indirect molecular biomarkers have been studied in relation to exercise (Appendix: Supplementary Table S1). To enable critical interpretation of biomarker data, it is important to identify methodological issues and variables that can help explain the disparities between the effects observed in different studies.

Methodological concerns and confounding factors when studying exercise effects on SF biomarker profiles

The importance of intensity and duration of exercise emerges from several studies, as biomarker changes seem to occur mostly under conditions of high intensity and long-term training. In horses, this was reflected by an increase in levels of C1, 2C, COL CEQ, CPII, GAG, CS846 and PGE2 (Frisbie et al., 2008). In dogs and rabbits, a similar effect was found for levels of COMP, MMP-1 and -3 and TIMP-1 (Qi and Changlin, 2006; Qi et al., 2007). However, the contribution of exercise intensity and duration is not always clear. For example, in two studies, a mild to moderate intensity protocol was used for the training period, after which a last exercise bout with a higher intensity was performed prior to a single SF collection procedure within 30 min or 1 h after exercise (Van den Hoogen et al., 1998; Brown et al., 2007). This approach makes it impossible to discriminate between the direct contribution of a single, high intensity exercise challenge and the effects of long-term, moderate-level training. Thus, any exercise regimen should be well defined and unambiguous.

Apart from the intensity and duration of exercise, several other factors could confound the relationship between SF biomarkers and exercise. Importantly, disparate effects have been observed for different joints, as evidenced by the exercise-induced increase in chondroitin sulphate in the tibiotarsal but not the radiocarpal joint (Lamprecht and Williams, 2012). Secondly, exercise history might influence effects of exercise on biomarker changes. For example, in polo ponies, a significant increase in hyaluronic acid concentration during the season was demonstrated in young ponies (3 to 4 years old) starting their careers, but not in older ponies (10 to 16 years old) with a career spanning 5 years or longer (Baccarin et al., 2014). There might have been an age-related adaptation effect, as the younger ponies showed an initial increase followed by a more or less steady state, whereas in the older ponies, a non-significant initial rise followed by a decline back to baseline was observed. Similarly, a canine study showed an overall rise in COMP levels within a period of 10 weeks of exercise, consisting of an increase from week 2, a peak at week 4, and a decline after week 6 that continued until the end of the study (Qi and Changlin, 2006). These observations stress the importance of frequent sampling, especially when aiming to evaluate biomarker dynamics over a longer period.

The moment of sampling after completing an exercise session is also of great importance. Increases in PGE_2 and TNFα were measured at 3 and 6 h (PGE_2) and at 2 h (TNFα) after exercise, and these biomarkers presented a return to baseline levels within 6 to 24 h (PGE_2) and within 12 h (TNFα), suggesting recovery of joint homeostasis (van den Boom et al., 2005; Baccarin et al., 2014). Therefore, the interval between the end of an exercise session and SF collection should be specified and standardised during the study period. In this context, the influence of repeated arthrocentesis also

needs to be taken into consideration, as SF levels of PGE_2, CS, NO and MMP activity can be affected for 2 to 60 h following the previous joint aspiration (Brama et al., 2004; van den Boom et al., 2004, 2005; Lamprecht and Williams, 2012; Baccarin et al., 2014). The time frame varies with each marker, as one study demonstrated that GAG levels were not affected at 12 h after arthrocentesis, but after another 60 h, there was a significant increase; this effect disappeared 7 days later (van den Boom et al., 2005).

Pathologic conditions such as the development of OA, which manifest clinically as lameness and which might or might not be exercise-induced, are important factors that can influence the SF biomarker profile and are additional to the effect of exercise. Decreased COMP concentrations correlating with an increase in total days of training were seen in lame horses but not in sound horses in one study (Skiöldebrand et al., 2006). In another study, an observed significant increase in CS concentration in young polo ponies was mainly due to three animals that developed OA within 24 months after the end of the study (Baccarin et al., 2014).

Another confounding factor in SF biomarker analysis is joint effusion resulting from high intensity exercise or from exercise-induced pathology. Due to dilution effects, apparently decreased biomarker levels can occur, despite increased absolute synthesis. Reporting marker ratios (e.g. MMP/TIMP, CPII/C2C, CS-846/GAG) rather than absolute concentrations of single markers might circumvent bias due to dilution effects (Qi and Changlin, 2006; de Grauw et al., 2011), but this approach assumes equal clearance rates of each marker from the joint space. In reality, individual markers present wide variations in SF clearance rates (Simkin, 1995).

As a last precaution to interpretation of SF biomarker data, it should be noted that when conducting biomarker research in equine SF, it is imperative to use analytic techniques that have been validated for use in this sample matrix and this species. It goes without saying that failure to do so can lead to severe misinterpretations of results.

Conclusions

Exercise is a prerequisite for the maintenance of joint homeostasis and plays a crucial role in the physiologic maturation process of the juvenile joint. Moreover, exercise has a major influence on the delicate homeostatic balance within the articular tissues and on their interplay, which is crucial for proper and durable joint function. However, there appears to be a fine line between the beneficial and detrimental effects of exercise on joint homeostasis, and the amount and intensity of exercise potentially has a lasting effect on tissue characteristics in juvenile animals, and affects joint homeostasis in mature animals. Physical overload disrupts the integrity of joint structures, resulting in inflammatory responses, hence affecting joint homeostasis. Such disturbances of joint homeostasis have negative effects on chondrocyte vitality and responses, and can eventually lead to irreparable damage to articular cartilage. However, the appropriate amount of exercise could stimulate the restitution of joint homeostasis and recovery of joint function, by stimulating proteoglycan synthesis and by promoting circulation within the various joint components. Biomarkers in SF can provide real-time information on the effects of exercise on joint homeostasis in in vivo situations, but the complexity of joint physiology and many other confounding factors, including the type of exercise, exercise history, timing of sampling and sampling intervals, joint type and concomitant pathology, complicate the interpretation of results and comparisons between studies.

Conflict of interest statement

None of the authors has a financial or personal relationship with people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2017.03.004.

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