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Responsiveness of BOLD MRI to Short-Term Temperature Changes in Rabbit Knees with Inflammatory Arthritis

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Abstract

Objectives: Tissue signal change caused by temperature-dependent alterations in the affinity of oxygen for hemoglobin contributes to BOLD contrast. Our goals were to test whether BOLD MRI contrast changes with short-term variations in joint temperature (internal responsiveness) and to determine whether an association exists between BOLD MRI and intraarticular pO_2 and blood flow [reference standards] upon joint temperature changes (external responsiveness).

Methods: Seven juvenile rabbits had the carrageenin (antigen)-injected knee imaged, 10 had the contralateral knee imaged and 11 had one of non-injection knees imaged. Assigned knees underwent experimentally forced increased (37°-42°C) or decreased (37°-30°C) local temperature for 15-minute periods using heating/cooling pads and were subsequently imaged. Intra-articular pO_2 and blood flow were measured *in vivo* during MRI scanning by polarographic probes.

Results: Relative BOLD MRI measurements showed moderate (≤0.8 and >0.5) or large (>0.8) Standardized Response Mean (SRM) changes upon increased local temperature in contralateral knees. Significant substantial correlations were obtained between absolute BOLD MRI measurements and intraarticular pO_2 measurements in contralateral knees (r = 0.71, *P* = 0.048) upon increased local temperature and high correlations were obtained in arthritic knees (r = 0.92, *P* = 0.004) upon decreased local temperature.

Conclusions: BOLD MRI was responsive to short-term joint temperature changes. Imaging contralateral joints in addition to arthritic joints with BOLD MRI may help maximize identification of minimal early changes in subjects with arthritis if findings of this study are confirmed in larger sample size experiments.

Keywords: Inflammatory arthritis; Rabbits; Responsiveness; BOLD MRI; Polarographic probes; Knees

Introduction

Juvenile idiopathic arthritis (JIA) is the most common connective tissue disease in the pediatric population with a prevalence of approximately 1 in 10,000 children in North America [1]. This chronic disease is associated with inflammation of the synovium which is a highly vascularized membrane that overlies the articular cartilage of the joints. The synovium inflammation leads to hyperproliferation of synovial cells and activation of the pro-inflammatory cytokine cascade, leukocyte infiltration, tissue damage and osteochondral destruction. An early event in inflammatory arthritis is an alteration in blood vessel density as a result of neovascularization (angiogenesis) [2]. Hyperplasia of the synovium necessitates a compensatory increase in the number of blood vessels to nourish and oxygenate the tissue. However, angiogenesis may not keep pace with synovial proliferation, leading to regions of hypoperfusion and hypoxia [2]. Later on during the disease course this highly vascular synovium can undermine the articular cartilage and erode bone at the osteochondral junction resulting in joint deformity and severe functional impairment if the disease is not treated in its early stage [2].

Physiologic changes tend to precede anatomic and clinical events in tissues [3]. Changes tend to precede anatomic and clinical events in other tissues. Early assessment of the physiologic changes that occur during the progress of inflammatory arthritis is much needed to enable early intervention prior to the development of irreversible changes in the joints which can currently be detected with anatomic MRI. So far only anatomic MRI outcome measures are available in clinical trials of juvenile [4] and adult rheumatoid arthritis [5-7]. There is a clear need for the development of novel functional imaging outcome measures for inflammatory arthritis.

Blood Oxygen Level Dependent (BOLD) MRI is a functional technique that is sensitive to the microvascular environment through fluctuation in the oxyhemoglobin to deoxyhemoglobin ratio [8] and that holds potential as a functional outcome measure for assessment of inflammatory arthritis. Oxyhemoglobin is diamagnetic, whereas deoxyhemoglobin is paramagnetic, which produces a local bulk magnetic susceptibility effect and subsequent MRI signal change typically observed in $T(2)^*$-weighted functional MRI scans [9,10]. It is well known that tissue signal change caused by temperature-dependent alterations in the affinity of oxygen for hemoglobin contributes to BOLD contrast because it is partly sensitive to the amount of paramagnetic deoxyhemoglobin in the voxel [11].

Outcome measures in clinical trials should be reliable, valid and responsive [12-14]. Previously studies have demonstrated the reliability of the BOLD MRI technique for data acquisition [15] and interpretation [16] in a rabbit model of inflammatory arthritis and provided an insight towards the validity of the BOLD MRI technique for assessment of inflammatory arthritis in a rabbit model at 1.5 Tesla [17]. Responsiveness is the extent to which measures are able

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to represent change over time [18]. To our knowledge no previous study has investigated the responsiveness of BOLD MRI to short-term changes of local joint temperature, similar to what occurs in arthritis, in a rabbit model of inflammatory arthritis.

In this study our hypothesis was that in acute arthritis the local temperature, blood flow, and oxygen consumption would all increase which would lead to an elevated oxygen extraction by the hypoxic perisynovial tissues. These physiologic changes would induce a consequent substantial decrease in BOLD signal contrast if no or poor compensatory mechanisms for local hypoxia were in place or alternatively to a mild decrease in BOLD signal contrast if such physiologic mechanisms had already partially compensated for the local hypoxia. The BOLD signal changes would subsequently return to baseline levels. This effect would be intensified by experimentally increasing the local joint temperature (more in arthritic than in non-arthritic joints) and would be reversed by introducing an intervention that aimed to decrease the local joint temperature.

The objectives of this study were therefore two-fold: (1) to test whether BOLD MRI values change with increased (37-42°C) and decreased local joint temperatures (37-30°C) in relation to corresponding baseline values over a short period of time (15 minutes) [internal responsiveness]; and (2) to determine whether an association exists between BOLD MRI values (test) and results of intraarticular pO2 and blood flow (BPU) [reference standard measures] with increased and decreased local joint temperatures over a short period of time (15 minutes) [external responsiveness]. Internal responsiveness characterizes the ability of a measure to change over a particular prespecified time frame. External responsiveness reflects the extent to which changes in a measure over a specified time frame relate to corresponding changes in a reference standard measure of health status [18].

Methods

The study protocol was approved by the Animal Care Committee of our institution and complied with the guidelines of the Canadian Council for the Protection of Animals.

Experimental design

Twenty-eight male juvenile New Zealand white rabbits, aged 10 weeks at the time of the experiment, were used in this study. The study had two phases. In phase 1 of the study **(*in-vivo* experiments outside the MRI scanner)** each of the select (n = 4) non-injection rabbits underwent both temperature increase and decrease procedures to determine the extent of time it would take for physiological blood parameters of the rabbit synovial tissue to reach a steady state as the temperature of the joint was experimentally forced to change. In phase 2 of the study **(*in-vivo* experiments inside the MRI scanner)** 28 rabbits were randomly assigned to injection (arthritic or contralateral) or non-injection (control) groups. In the injection group (n = 17 rabbits, 61%) either the arthritic (n = 7, 25%) or the contralateral knee (n = 10, 36%) was assigned for the study. In the non-injection group (n = 11 rabbits, 39%, 4 of those were also used in experiments of phase 1) none of the knees received any injections and only one knee per rabbit was assigned for the study (Figure 1). Injection group rabbits had one of their knees injected with carrageenin as described elsewhere [19] the day prior to imaging in order to generate an inflammatory response to simulate acute arthritis (injection group). In both phases 1 and 2 of the study each rabbit had an angiocatheter placed into one of the knee (hind limb) joints. Because we were interested in evaluating the responsiveness of BOLD MRI to short-term variations in joint temperature both related (arthritic joints) and not related (non-arthritic joints) to early synovitis we used an animal model of acute arthritis as well as control animals. Because we aimed to assess these changes in the limbs of a rabbit model that best corresponded to human knees, which are weight-bearing joints, we elected to evaluate the hind limbs of rabbits which have major weight-bearing biomechanics in this animal model.

In-vivo experiments outside the MRI scanner

For the experiments the animals were anesthetized intravenously with an injection of 11-15 mg of ketamine hydrochloride (Ketalar; Parke-Davis, Morris Plains, NJ)/kg of body weight and 1.1 mg/kg of xylazine hydrochloride (Rompun; Miles, Shawnee, Kan), and placed in supine position. The angiocatheters were placed in such a position in the knee (hind limb) joints that allowed subdermal polarographic probes attached to an OxyLite/OxyFlow system (Oxford Optronix Ltd., London, England) to make readings in the joint. This enabled the calculation of blood flow (BPU) and oxygen partial pressure (pO_2), which were reference standards for BOLD measurements. OxyLite provided absolute values for pO_2 in mmHg by using Fluorescence-Quenching Technology [20]; and OxyFlow provided continuous measurements of tissue blood flow in arbitrary blood perfusion units (BPU) by using Laser Doppler Flowmetry [21]. Additional temperature probes as part of the aforementioned system provided real time tissue measurements according to local increase and decrease of temperature.

The temperature of the joint was forced to increase (at least 40°C up to a maximum of 42°C) or to decrease (down to a minimum of 30°C), and the readings of blood perfusion and oxygen partial pressure were monitored and recorded. The temperature of the joint was changed to the desired value, and kept there for as long as it took for the tissue blood parameters to stabilize for at least 15 minutes. The temperature was then set back to baseline (37°C), and recording of the parameters

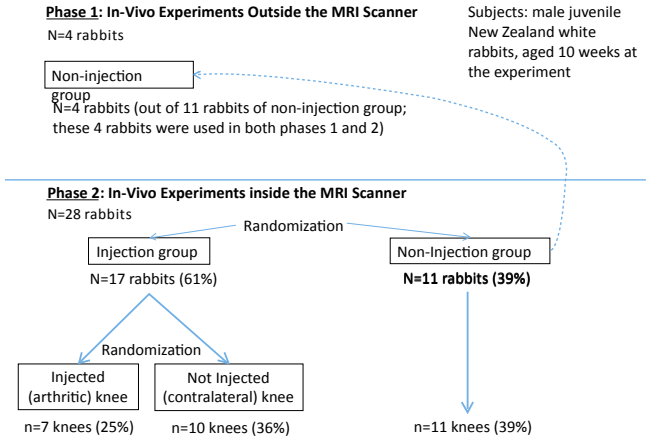


Figure 1: Flow diagram of the experimental protocol of the study. The purpose of phase 1 was to determine the extent of time it would take for physiological blood parameters of the rabbit synovial tissue to reach a steady state as the temperature of the joint was experimentally forced to change. The devices used in this phase were intraarticular angiocatheters for measurement of blood perfusion (BPU), oxygen partial pressure (pO2) [reference standard measures) and intraarticular temperature at forced increased and decreased joint temperatures. The purposes of phase 2 were to test whether BOLD MRI values change with increased (37-41°Celsius) and decreased local joint temperatures (37-32°Celsius) in relation to corresponding baseline values over the period of time established in phase 1 in 3 different types of joints (arthritic and contralateral knees of injection group rabbits and control knees of non-injection group rabbits) [internal responsiveness]; and to determine whether an association exists between BOLD MRI values (test) and results of intraarticular pO2 and BPU [reference standard measures]. Only one knee per rabbit underwent the temperature change procedures.

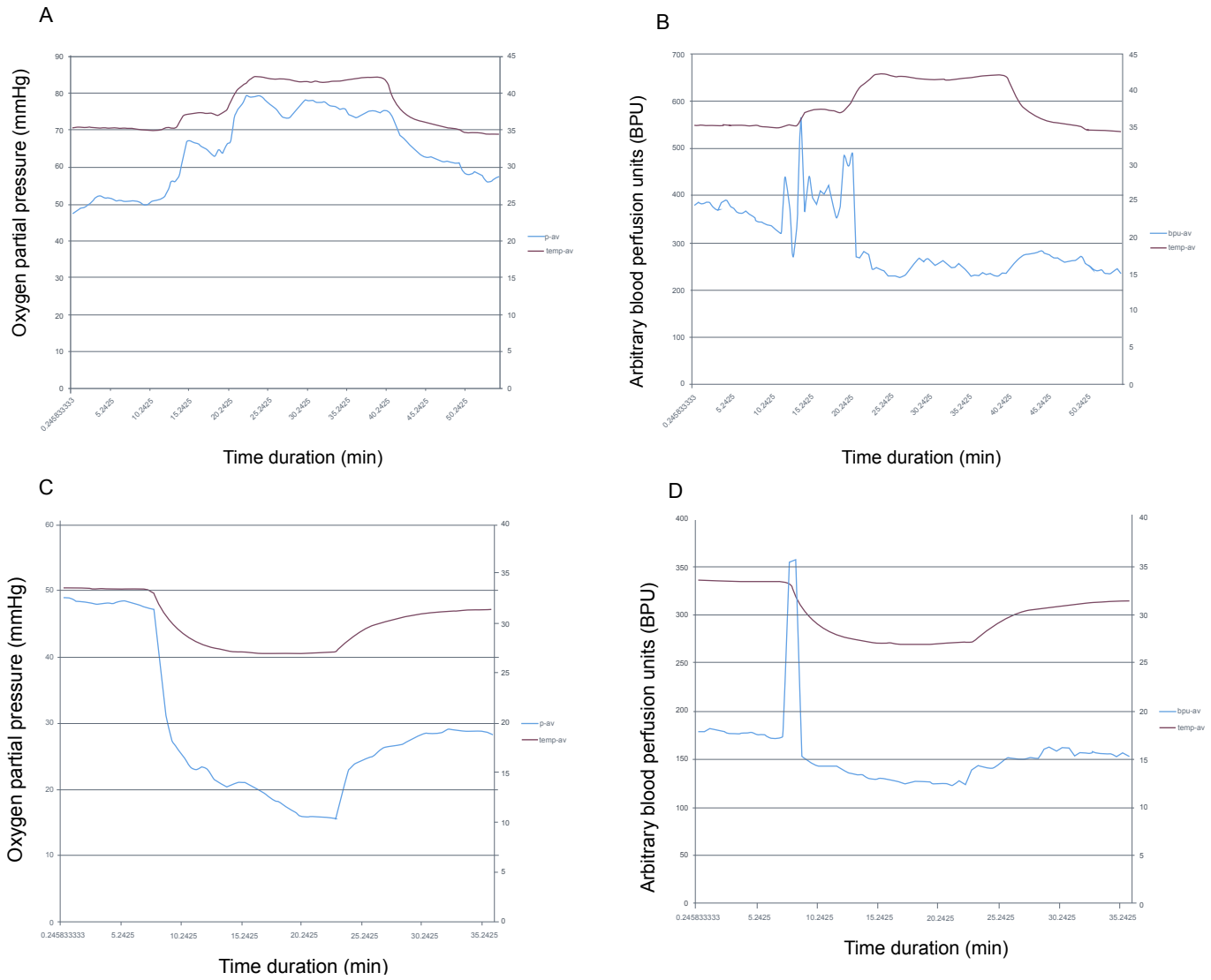


Figure 2: Plots of *in vivo* experiments conducted outside the MRI environment show the readings of oxygen partial pressure (**A, C**) and blood flow (**B, D**) when the temperature of the joint was steadily changed to a desired value (at least 40°C but not higher than 42°C (for increased temperature) and 32°C, but not lower than 30°C (for decreased temperature), and kept there for as long as it took for the tissue blood parameters to stabilize within reason. The temperature was then set back to normal, and recording of the parameters of interest was again maintained until the parameters stabilized. Please note that it took at least 15 minutes for the physiologic response of pO2 (**A**) or BPU (**B**) to increase to the desired point and remain stable at that point, but less than 10 minutes for the pO2 (**C**) or BPU (**D**) measurements to decrease to the desired point and remain stable at that point. Once a plateau on pO2 and BPU measurements was reached for increased (**A, B**, respectively) and decreased (**C, D**, respectively) local temperature it took approximately 15 minutes for pO2 and BPU measurements to respond to the local temperature forced change towards baseline values.

of interest was again maintained until the parameters stabilized. This procedure was repeated for both an increase and decrease in the joint temperature. The plots of increased and decreased temperature, blood perfusion, and oxygen partial pressures were then analyzed (Figure 2).

In-vivo experiments inside the MRI scanner

Following anesthesia as described in the prior session on experiments outside the MRI scanner the hind legs were extended to create an approximately 120° angle between femur and tibia, and then tied together and to a cushion to maintain the position. Angiocatheters were placed intra-articularly in both knees and attached to the OxyLite/OxyFlow system.

Imaging data acquisition

The rabbits were imaged in a 1.5 Tesla MRI magnet (Excite III 12.0 TwinSpeed MR unit, General Electrics Medical Systems, Milwaukee, WI) with a 3 inch receive-only surface coil. Extension cables for the probes were used so that the system would be MR compatible. After anatomical scans (T_1-weighted 2D spin-echo sequence; 7 contiguous axial slices; 10 cm field of view, 3 mm-thick; TE/TR = minimum/500 msec; 15.63 bandwidth; 1 NEX; 256 frequency and 256 phase, matrix; 2:16 scan time) functional images (spiral 2D sequence; 7 contiguous axial slices; 10 cm field of view; 3 mm-thick; TE/TR = 40/2000 msec; 90° flip angle; 64 frequency, matrix; 6:14 scan time) of both joints were simultaneously acquired by giving the animal alternating normoxic (ambient air, 21% oxygen) and hyperoxic supplies of gas (95% oxygen

mixed with 5% carbon dioxide, carbogen, at 60 second intervals) through a face mask. A T_2^*-weighted gradient-echo imaging sequence with a single shot spiral readout [22] was applied over the same acquisition volume as T_1-weighted scans. This scan acquired 45 spatial slices, from the inferior landmark upwards (TE/TR = 40/2000 msec, 2 NEX, 64 freq, 62 kHz bandwidth, 90° flip angle, 1 spiral interleaf containing 4,096 points regridded to 64×64 point acquisition matrix, scan time = 3:04 min).

BOLD scans were obtained at baseline (37°C) temperature and then when the temperature of one of the knees reached a steady state towards a prior determined ceiling and floor temperatures. A heating/cooling pad placed around the knee and connected to a water circulator [Refrigerating / Heating Circulator, Standard Model 9106, PolyScience, Preston Industries Inc., Niles, IL] (Figure 3) was used to maintain the temperature above 40°C during the course of scanning, while attempting to remain not higher than 42°C (sequence of 37°C to 42°C), and below 32°C, while attempting to remain not lower than 30°C (sequence of 37°C to 30°C). The temperature of the joint was monitored using the readings of the OxyLite system.

At the end of the imaging procedure the rabbits were euthanized with an intravenous overdose of pentobarbital (Nembutal; Abbott Laboratories, North Chicago).

Imaging data analysis

The BOLD MRI data was processed and analyzed using the software program Stimulate (University of Minnesota, MI) [16]. The program performed boxcar cross-correlation to determine maps of activation of image voxels with the hyperoxic stimulus. The maps of BOLD signal difference (hyperoxia – normoxia) were expressed as a percentage of baseline signal (PT%) and a corresponding map of the Pearson's r-correlation value (on_off) indicating the fit of each voxel's time-course data to the hyperoxia time-course model. Free-hand ROIs were drawn by a single operator (G.N.) using pre-defined anatomic landmarks [15]. Only those voxels with a positive BOLD signal difference and with r-values above one of two thresholds were selected for further analysis. One threshold (r>0.2) was chosen to control the expected proportion of false positive voxels to <0.02. A much more lenient threshold of r>0.01 was also used for comparison. The percentage of suprathreshold voxels within the region-of-interest (ROI) [PT%]) and the mean of the percentual BOLD signal differences (on_off) within the selected voxels were used as the summary BOLD measures for each investigated knee at increased and decreased local temperature experiments (Figure 4 and Figure 5). Four combinations of MRI parameters sets that were shown to have acceptable reliability in a previous study [15] were used for data analysis as shown below:

Data acquisition	Data analysis
spiral; TE = 40, 95% O2+5% CO2,60 sec	diff on_off_0.2
spiral; TE = 40, 95% O2+5% CO2, 60 sec	PT%_0.2
spiral; TE = 40, 95% O2+5% CO2, 60 sec	diff on_off_0.01
spiral; TE = 40, 95% O2+5% CO2, 60 sec	PT%_0.01

*__Abbreviations:__ TE: Echo-Time; sec: Seconds; diff_on_off: Difference in on and off changes; PT%, Percentage of Activated Voxels

Note: The paradigms that included PT% or diff on_off_0.2 and PT% or diff on_off _0.01 in the data analysis represented the percentage of suprathreshold voxels or the mean of the percentual BOLD signal differences (on_off) within the selected voxels when thresholds >0.2 and >0.01 were applied.

Statistical analysis

Differences in absolute BOLD MRI measurements at baseline between different categories of knees were analysed with analysis of variance (ANOVA).

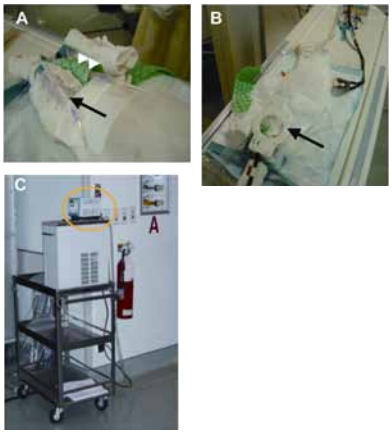


Figure 3: Angiocatheters were placed into the synovium of the knees (**A**, arrow), such that subdermal polarographic probes attached to an OxyLite/ OxyFlow system (Oxford Optronix Ltd., London, England) could make intraarticular readings of blood perfusion (BPU), oxygen partial pressure (pO2), and temperature of the tissue in real time according to local increase and decrease of temperature. The functional scans were acquired while the temperature of one of the knees was increased, and again when it was decreased using a 3 inch receive-only surface coil (**B,** arrow). A heating/ cooling pad placed around the knee (**A**, arrowheads) and connected to a water circulator (**C**) was used to maintain the temperature above 40°C during the course of scanning, while attempting to remain not much higher than 42°C, and below 32°C, while attempting to remain not much lower than 30°C. The temperature of the joint was set using a temperature selector (**C**, circle) and was monitored using the readings of the OxyLite system.

We calculated differences in percentual BOLD MRI signal changes with increased and decreased local joint temperatures in relation to corresponding baseline values (prior to induced local temperature changes) between arthritic and contralateral or arthritic and non-injection knees using Student t-tests. Values were provided as relative (BOLD measurement at increased or decreased local joint temperature minus BOLD measurement at baseline/BOLD measurement at baseline) values.

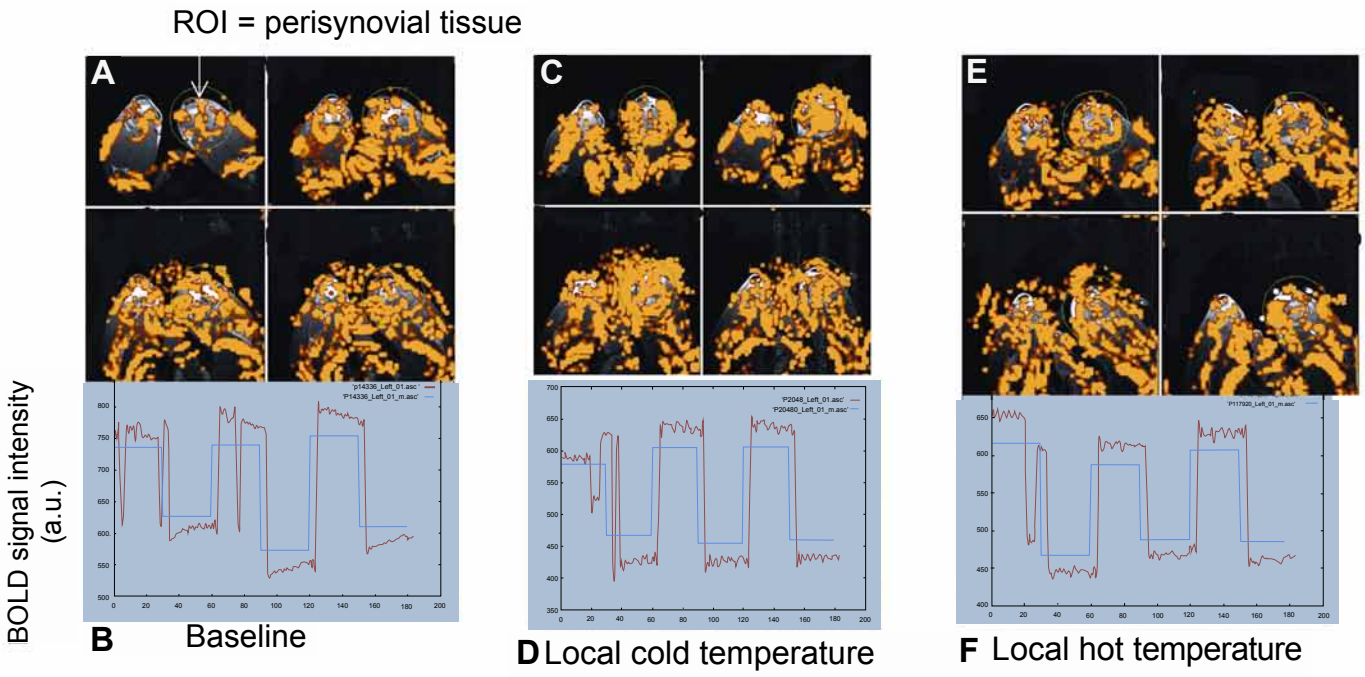
Internal responsiveness of BOLD MRI measures was determined by calculating the Standardized Response Mean (SRM). SRM is the ratio of observed change and the standard deviation reflecting the variability of the change values (SRM = difference between the mean baseline values and follow-up values/standard deviation of this difference) [6]. We hypothesized that BOLD MRI measurements would suffer moderate changes (standardized response mean >0.5 and ≤ 0.8) upon forced joint temperature changes. Values of >0.8 change were considered to represent large changes, ≤0.8 and >0.5 moderate changes, and ≤0.5 and >0.2 small changes [23,24].

External responsiveness was assessed by measuring Pearson's correlation coefficients (r-values) [25] between BOLD MRI measurements and corresponding intraarticular pO2 and BPU measurements at increased and decreased local joint temperatures. *R*-values ≤ 0.40 indicated poor, >0.40 and ≤0.60 moderate, >0.60 and ≤0.80 substantial and >0.80 excellent agreement [26]. Statistical significance was reached at alpha <0.05.

Results

In-vivo experiments outside the MRI scanner

From the separate experiments of induced knee temperature



Abbreviations: ROI: Region-of-Interest; a.u: Arbitrary Units.

Figure 4: Mapping and time course of the response in T2* signal during respiratory challenge (carbogen stimulus for data acquisition, 0.01 threshold for data analysis [**A-F**] in the carrageenin-injected (arthritic) knee of a rabbit model. Panels B, D, F show changes in the on-and-off signal intensity in the corresponding region-of-interest for 60-second interval paradigms of normoxia (off) and hyperoxia (on) at the time of the experiment. The red lines of the graphs indicate the averaged time courses of differences in BOLD signal intensity in the perisynovial tissue that were measured and the blue lines the superposed on- and –off Talairach-based paradigms that were used for calculations.

change outside of the MR scanner it was shown that the lag time between a forced local decrease in temperature and the physiological response (i.e. corresponding change in pO_2 or BPU) in the beginning of the experiment was shorter (approximately 10 minutes) than the usual elapsed time between temperature stabilization upon forced change and return of physiologic measurements of pO_2 or BPU to baseline values later on during the experiment. With regard to forced local increase in temperature approximately 15 minutes of lag time were needed for the physiologic response of pO_2 or BPU to increase to the desired point and remain stable at that point and to return to baseline values once a maximum plateau was reached (Figure 2). Based on these preliminary experiments we accepted 15 minutes as an appropriate lag time between the start of forced local temperature change and the start of MR imaging acquisition.

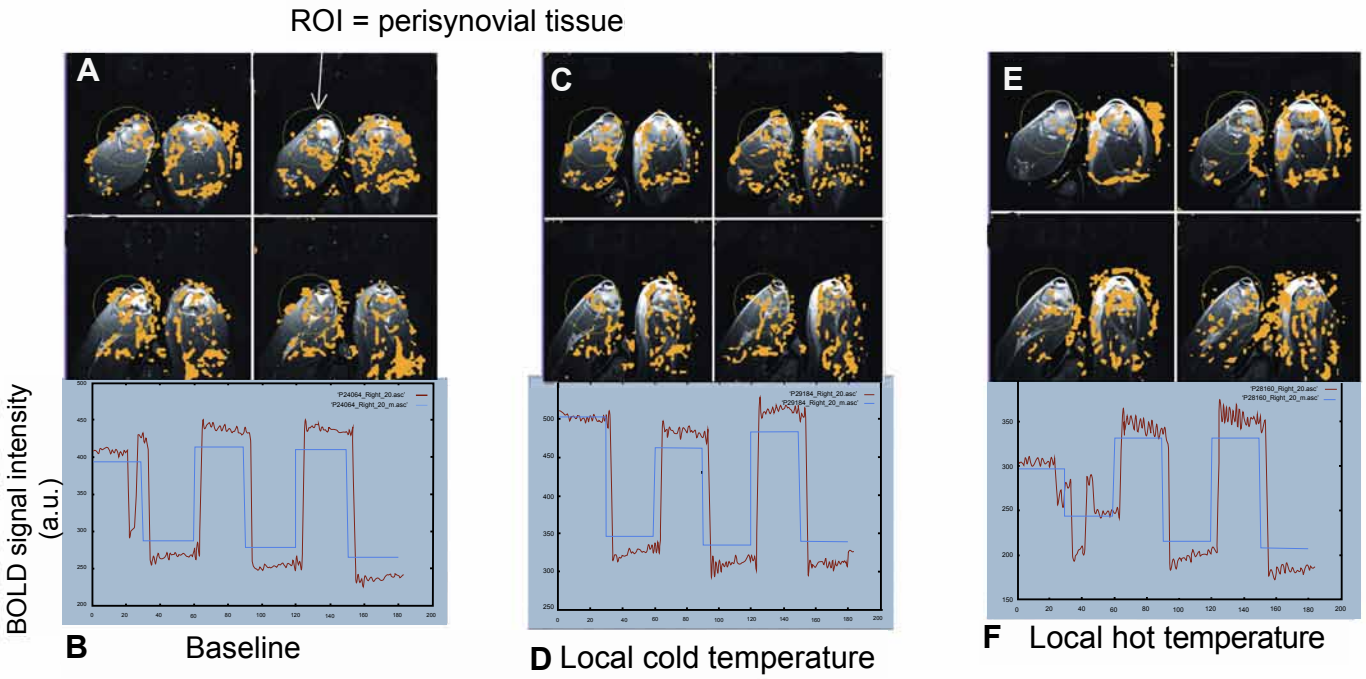
In-vivo experiments inside the MRI scanner

Internal responsiveness: At baseline, absolute BOLD MRI measurements had mean values ± SD of 11.91 ± 6.81, 9.04 ± 6.04, 9.76 ± 5.53 (on_off_0.2) or 14.22 ± 8.69, 12.50 ± 7.62, 16.00 ± 1.67 (on_off_0.01) in groups of arthritic, contralateral and control knees.

A tendency towards statistically significant differences in percentual values of BOLD MRI signal change were noted for increased joint temperatures between arthritic and contralateral knees ($P = 0.08$, diff_on_off_0.2 and $P = 0.08$, PT%_0.2) and between arthritic and non-injection knees ($P = 0.09$, PT%_ 0.2) (Table 1). Nevertheless, in arthritic knees under increased local temperature there was a percentual loss in signal change by 9%-16% (= mean change range, -0.09 to -0.16 × 100) and under decreased local temperature a percentual increase in signal change by 1% (= mean change range, 0.01 × 100) or a percentual decrease by 0.05% (= mean change range, -0.005 × 100) [on_off_0.2 or 0.01] (Table 1). Thus, numerically when we increased the temperature of arthritic joints the percentual BOLD MRI signal change compared to baseline in these joints was *smaller* (lower BOLD signal change at increased local temperature than at baseline) than the percentual BOLD MRI signal change in the contralateral and non-injection joints. Conversely, overall when we decreased the temperature of arthritic joints the percentual BOLD MRI signal change compared to baseline in these joints tended to be numerically *greater* (greater numerical BOLD signal change at decreased local temperature than at baseline without reaching statistical significance between types of joints) than the percentual BOLD MRI signal change in the contralateral and non-injection joints (Table 1). In contralateral and control joints (on_off_0.2 or 0.01) regardless of an increase or decrease in the local temperature there was a percentual numerical decrease in signal change between baseline and the steady state. In contralateral joints for increased local temperature the range of signal change decrease was 2%-4% (= mean change range, -0.02 to -0.04 × 100) and for decreased local temperature, the range of signal change decrease was 3%-7% (= mean change range, -0.03 to -0.07 × 100). In control joints for increased and decreased local temperature the range of signal change decrease was 3% (= mean change range, -0.03 × 100) (Table 1).

The SRM for relative changes of BOLD MRI in inflammatory arthritis at increased local temperatures using PT% 0.2 the SRM for relative BOLD changes were large (SRM = 1.93) for contralateral knees and moderate (SRM = 0.54 and 0.56, respectively) for arthritic and control knees. Using PT% 0.01 the SRM values were large (SRM = 1.75) for contralateral knees (Table 2).

At decreased local temperatures, relative BOLD MRI measurements



Abbreviations: ROI: Region-of-Interest; a.u: Arbitrary Units; PT%: Percentage of Suprathreshold Voxels within the ROI

Figure 5: Mapping and time course of the response in T2* signal during respiratory challenge (carbogen stimulus for data acquisition, 0.2 threshold for data analysis [**A-F**] in the knee contralateral to the arthritic knee of a carrageenin-injected rabbit model. A large standardized response mean (SRM) in PT% was depicted between baseline (**A**) and short-term exposure to increased temperature (**C**). Panels B, D, F show changes in the on-and-off signal intensity in the corresponding region-of-interest for 60-second interval paradigms of normoxia (off) and hyperoxia (on) at the time of the experiment. The red lines of the graphs indicate the averaged time courses of differences in BOLD signal intensity in the perisynovial tissue that were measured and the blue lines the superposed on- and –off Talairach-based paradigms that were used for calculations.

demonstrated moderate responsiveness in contralateral knees using high (0.2) and low (0.01) thresholds (SRM = 0.73 and 0.75, respectively) for data analysis. Similar moderate responsiveness was noted in arthritic knees using high (0.2) thresholds (SRM = 0.58) as technical parameters (Table 3).

External responsiveness: Significant substantial correlations (r = 0.71, P = 0.048) were obtained between absolute BOLD MRI (on_off_0.2) measurements and intraarticular pO_2 measurements in contralateral knees upon increased local temperature. In arthritic knees however significant high correlations (r = 0.92, P = 0.004) between absolute BOLD MRI measurements (PT%_0.01) and intraarticular pO_2 measurements were only noted upon decreased local temperature. When the temperature was increased in arthritic knees, correlations between absolute BOLD MRI measurements (PT%_0.01) and pO2 (r = 0.68, P = 0.09) did not reach statistical significance.

No significant correlations between BOLD MRI measurements and intraarticular blood flow measurements were noted in any type of knee joints evaluated.

Discussion

We investigated the impact of experimentally-induced short-term temperature changes in joints on BOLD contrast at 1.5 Tesla. The results of this study indicate that from the perspective of SRM for internal responsiveness, relative BOLD MRI changes are largely sensitive to short-term changes of increased local temperature in knees contralateral to injected joints and moderately sensitive to these changes in arthritic and non-injection knees. With regard to decreased local temperature, relative BOLD MRI changes are moderately sensitive to these changes both in arthritic knees and in knees contralateral to injected joints. From the perspective of external responsiveness, BOLD MRI substantially correlates with intraarticular pO_2 measurements in contralateral knees upon increased local temperature, and highly correlates with intraarticular pO_2 measurements in arthritic knees upon decreased local temperature.

Despite the relatively small increase in the local temperature of an arthritic joint without any intervention (approximately 1°C) as noted in this study, previous studies [20] showed that body temperature changes ranging from 38° to 39°C result in increased BOLD signal in rat brains. In addition, it is well know that the metabolic rate increases by 6% for each 1°C increase in core temperature in rat brains [20], which may explain the small percentual BOLD changes noted in this study at 1.5 Tesla.

Results of this study showed that the arthritic status of a given joint may lead to a percentual loss in BOLD signal change compared to contralateral and control joints when the joint is exposed to a local hot stimulus confirming the hypothesis of this study.According to our hypothesis this would occur when compensatory mechanisms for local hypoxia were poor or were not in place at the time of the BOLD MRI measurements which is the expected scenario in most arthritic joints. Numerically, when the joint is exposed to a local cold stimulus an effect opposite to that expected in an arthritic joint is noted, i.e., a percentual increase in BOLD signal change was observed. These differences in BOLD signal change to short-term differences in local temperature could occur due to focal vasodilation (local hot stimulus) and vasoconstriction (local cold stimulus) at the joint level. They are expected to be more robust as a response to long-term local thermal

	Increased Temperature				Decreased Temperature			
	Arthritic	**Contralateral**	**Non-Injection**	**P-value**	**Arthritic**	**Contralateral**	**Non-Injection**	**P-value**
Diff_on_off_0.2, 95% O2+5% CO2, 60 sec	-0.09 (0.09)	-0.02 (0.06)	-0.03 (0.06)	*P* = .08 (arthritis vs contralateral); *P* = .20 (arthritis vs control)	0.01 (0.18)	-0.07 (0.48)	-0.03 (0.06)	*P* = 0.29 (arthritis vs contralateral); *P* = 0.86 (arthritis vs control)
PT% 0.2, 95% O2+5% CO2, 60 sec	-0.46 (2.26)	-0.02 (0.40)	0.11 (0.28)	*P* = .08 (arthritis vs contralateral); *P* = .09 (arthritis vs control)	0.26 (1.04)	-0.07 (0.37)	0.07 (0.32)	*P* = 0.35 (arthritis vs contralateral); *P* = 0.62 (arthritis vs control)
Diff_on_off_0.01, 95% O2+5% CO2, 60 sec	-0.16 (0.42)	-0.04 (0.16)	-0.03 (0.12)	*P* = .15 (arthritis vs contralateral); *P* = .34 (arthritis vs control)	-0.005 (0.41)	-0.03 (0.32)	-0.03 (0.19)	*P* = 0.87 (arthritis vs contralateral); *P* = 0.90 (arthritis vs control)
PT% 0.01, 95% O2+5% CO2, 60 sec	-0.35 (0.94)	0.09 (0.23)	0.12 (0.17)	*P* = .13 (arthritis vs contralateral); *P* = .13 (arthritis vs control)	0.03 (0.25)	0.008 (0.30)	0.15 (0.31)	*P* = 0.90 (arthritis vs contralateral); *P* = 0.52 (arthritis vs control)

P values represent comparisons of BOLD MRI signal changes between arthritic and contralateral; and arthritic and non-injection joints.
Percentual BOLD MRI signal changes are obtained using the formula: [(BOLD signal change at a given local temperature state minus BOLD signal change at baseline) / BOLD signal change at baseline] x 100.
Values are provided as relative (BOLD measurement at increased or decreased local joint temperature minus BOLD measurement at baseline / BOLD measurement at baseline) values.
A negative value represents a signal change loss between baseline and the given temperature state and a positive value represents a corresponding signal change increase.
Abbreviations: diff_on_off: Difference in on and Off changes; PT%: Percentage of Activated Voxels; sec: Seconds

Table 1: Mean (standard deviation) change in BOLD MRI measurements (=BOLD signal change at a given local temperature state minus BOLD signal change at baseline / BOLD signal change at baseline) in arthritic (injected), contralateral to injected joints and non-injection joints upon stimulus of increased and decreased local temperature using 4 different paradigms for BOLD MRI data acquisition and analysis.

effects such as those that occur in longstanding inflammatory arthritis.

In the *in-vivo* experiments outside the MRI scanner of this study, the lag time between the locally induced decrease in temperature in the beginning of the experiment and the corresponding physiological change in pO2 or BPU was approximately 10 minutes and between temperature/PO2 and BPU plateau and return of pO2 or BPU measurements to baseline values, approximately 15 minutes. Fifteen minutes of lag time were also needed for the physiologic response of pO2 or BPU to be observed, i.e. to increase to the desired point and remain stable at that point and to return to baseline values. Previous studies [27] showed that the core temperature of mice that were implanted squamous cell carcinoma takes approximately 20 minutes to decrease from 37°C to 30°C. The rapid reversibility of BOLD signal upon differences in local temperature has been previously demonstrated [11] and is attributed to unloading of hemoglobin in heat-producing tissues. In rat brains, although the blood-brain barrier may have an effect on the BOLD signal, with a temperature difference of 2.2°C BOLD signal is expected to change by approximately 6% [11].

From the perspective of SRM, relative moderate or large BOLD MRI changes were noted in contralateral knees only upon a forced increase in the local joint temperature. The SRM results obtained for relative BOLD MRI measurements upon an experimental decrease in the local joint temperature were distinct from the results obtained upon an experimental increase in local temperature. In the first instance (at increased local temperature, Table 2) moderate responsiveness of relative BOLD values were demonstrated both in arthritic and non-injection knees, whereas in the second instance (at decreased local temperature, Table 3) moderate responsiveness was noted in arthritic and contralateral knees.

Previous studies [28] showed the effect of induction of reflex vasodilation on the affected extremity when the contralateral extremity is wrapped in a warm washcloth. The clinical applicability of this physiologic effect in upper or lower extremities is to warm up a contralateral upper or lower limb in cases of discoloration of a given extremity due to umbilical arterial catheters' complications. This procedure would increase the skin temperature of the contralateral extremity eliminating then the need for removal or replacement of the catheter [29]. This effect occurs due to reflex vasodilation and increased local perfusion in the affected extremity when the contralateral extremity is warmed up and requires a mature autonomic nervous system [28]. No such an effect has been previously reported for a locally induced decrease in the skin temperature of a given extremity. This could however explain why BOLD MRI showed better internal responsiveness in arthritic or non-injection knees when the local joint temperature was artificially decreased rather than in contralateral knees as noted when the local joint temperature was experimentally increased.

If the concept of this study was translated into clinical practice, contralateral joints could be imaged either simultaneously using phase-array coils or separately using dedicated extremity coils. Although the cost for such strategy would rely on increasing the MRI scanning time (imaging each joint separately) or reducing the quality of imaging (imaging both joints simultaneously) this additional procedure might contribute to the early diagnosis of functional changes in inflammatory arthritis if the results of this preliminary study are confirmed in larger series. The continuous ability of reducing the MRI scanning time [30] with parallel MR imaging techniques may turn this possibility feasible in the pediatric population in the near future thus implementing the use of MRI to guide individualized therapy using biologic agents in JIA patients.

Results of this study showed that absolute BOLD MRI measurements correlate substantially with reference standard values of intraarticular pO2 measurements in knees contralateral to injected knees at increased local temperatures demonstrating the ability of external responsiveness of BOLD MRI to a locally increased temperature stimulus. This result supports the internal responsiveness findings of the study with regard

	BOLD MRI Parameters	
Knees	**Diff_on_off_0.2, 95% O2+5% CO2, 60 sec**	**Diff_on_off_0.01, 95% O2+5% CO2, 60 sec**
	Standardized Response Mean	
Arthritic	-0.22 – Small	0.03
Contralateral	-0.48 – Small	0.07
Non-injection	-0.01	0.40 – Small
Knees	**PT% 0.2, 95% O2+5% CO2, 60 sec**	**PT% 0.01, 95% O2+5% CO2, 60 sec**
	Standardized Response Mean	
Arthritic	0.54 – **Moderate**	0.16
Contralateral	1.93 – **Large**	1.75 - **Large**
Non-injection	0.56 - **Moderate**	0.40 – Small

Abbreviations: diff_on_off: Difference in on and Off changes; PT%: Percentage of Activated Voxels

Table 2: Standardized response mean for assessment of internal responsiveness of relative BOLD MRI measurements at increased local temperatures using different sets of BOLD MRI parameters in arthritic, contralateral and non-injection knees.

	BOLD MRI Parameters	
Knees	**Diff_on_off_0.2, 95% O2+5% CO2, 60 sec**	**Diff_on_off_0.01, 95% O2+5% CO2, 60 sec**
	Standardized Response Mean	
Arthritic	0.45 - Small	0.41 – Small
Contralateral	-0.12	0.03
Non-injection	-0.48 - Small	0.38 - Small
Knees	**PT% 0.2, 95% O2+5% CO2, 60 sec**	**PT% 0.01, 95% O2+5% CO2, 60 sec**
	Standardized Response Mean	
Arthritic	0.58 - **Moderate**	0.04
Contralateral	0.73 - **Moderate**	0.75 - **Moderate**
Non-injection	0.37 - Small	0.23 - Small

Abbreviations: diff_on_off: Difference in on and Off changes; PT%: Percentage of Activated Voxels

Table 3: Standardized response mean for assessment of internal responsiveness of relative BOLD MRI measurements at decreased local temperatures using different sets of BOLD MRI parameters in arthritic, contralateral and non-injection knees.

to moderate SRM changes noted in contralateral knees upon an experimental increase in the local temperature (best represented by PT %). Interestingly, however, absolute BOLD MRI measurements highly correlated with intraarticular pO_2 measurements in arthritic knees at locally decreased temperatures only. We hypothesized whether the ability of BOLD MRI to provide a quantitative measurement of the evolution of pO_2 in the tissue could be impaired or reduced if the temperature of an already warm arthritic joint was artificially increased. Previous studies [31] demonstrated that although a good temporal correlation can be noted between the increase in BOLD signal intensity and the increase in oxygenation status of a tissue, the local BOLD response to the variation of BOLD signal intensity cannot be related quantitatively to the response of pO_2.

Limitations of this study include a potential inaccuracy of the reference standard measures given differences in half-lives of polarographic probes and heterogeneity of synovium generating different BOLD signal in different parts of the synovium according to the ROI chosen for evaluation. Also, the polarographic probes were inserted blindly respecting anatomic landmarks and may have not reached the desired location in different rabbits' knees. Finally, a relatively high percentual loss in BOLD MRI signal change was noted in arthritic knees upon increased local temperature using PT% (range, 35%-46%, Table 1). This demonstrates that the PT% summary estimate used in this study has likely overestimated real physiologic joint changes (as compared with on_off changes in corresponding groups of joints) as per the nature of these measurements which only provide information on suprathreshold activated voxels.

Conclusion

BOLD MRI contrast is sensitive to short-term variations in joint temperature in an animal model of inflammatory arthritis. As a result, this novel imaging technique can potentially be of value as an outcome measure for follow-up of inflammatory arthritis in humans if the results of this experimental study are confirmed in longitudinal clinical trials.

The results of this preliminary study showed that BOLD MRI contrast is sensitive to short-term variations in joint temperature in an animal model of inflammatory arthritis. An observation of the study is that BOLD MRI changes can be appreciated in the knee contralateral to the injected knee. This observation raises the point that imaging non-affected joints contralateral to the arthritic could potentially be considered as an attempt to maximize the identification of minimal early changes in JIA. This is a new concept in functional imaging of joints based on the pre-existing knowledge on autonomic nervous reflex mechanisms for auto-regulation of temperature of joints. Nevertheless, the concept of increasing the local temperature of a given extremity by adding a heating pad / bandage to the contralateral extremity is widely accepted in clinical practice and is currently used in NICU units of large academic hospitals around the world. Nevertheless, further scientific investigation using a larger sample size and human subjects is required to confirm and validate the results of this preliminary study.

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