Imaging displacement and strain in the medial gastrocnemius muscle during ankle-joint motion using 2D-ciné DENSE MRI

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Declaration

I, Andrew James Lawson, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. This work has not been published prior to registration for the above mentioned degree.

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Introduction

Problem description

Skeletal muscle structure has been defined on both macro and microscopic levels by gross dissection, light- and electron-microscopy. The basic physiological building blocks involve the electromechanical coupling between interlinking actin and myosin fibres. Detailed intramuscular behaviour during contraction can be clearly defined when examining a single isolated muscle. However, there are few areas in the human body where single muscles act independently to affect motion. This thesis attempts to address the compounded effect that muscles have on each other, while working synergistically in a group, such as the calf muscle.

Modern imaging and biomechanical modelling have proposed a more detailed dynamic behaviour than uniformly shortening fibres when complex muscle groups work together to alter joint orientation. Ultrasound and Magnetic Resonance Imaging (MRI) are the two most common techniques available for imaging dynamic skeletal muscle motion. In this study, we apply both MRI displacement encoding and ultrasound to assess medial gastrocnemius tissue biomechanics during plantar flexion. An MRI sequence called Displacement Encoding with Stimulated Echoes (DENSE), is used to obtain maps of detailed displacement and deformation of the medial gastrocnemius muscle. These data are compared to results from ultrasound and are used to glean insights into the behaviour of a single muscle working within a complex group.
We anticipate that this muscle will be subject to effects from the remaining muscles comprising the calf belly.

A reference range of normal strain values is needed for non-injured calf muscle to provide comparative data for strain imaging of injured muscles. The ultimate aim would be in part to evaluate the injured muscle, but also to interrogate events in neighbouring muscles during the rehabilitation process, thereby preventing inadvertent secondary injuries. Strain imaging may also in future represent a method for objectively determining whether a sports injury has completely healed.

Scope and limitations

This work stems from a collaboration of specialists in the field of clinical radiology, MR physics and biomechanics. As such, the content of this thesis is of a broad scope. We assume that the reader understands the basic principles of skeletal muscle physiology, musculoskeletal MRI and ultrasound. For the clinical case studies, background material on muscle biomechanics and the principles of mechanical strain analysis are presented.

The MRI results presented in this thesis are from a sample of five normal volunteers. MRI-derived strain patterns in the medial gastrocnemius were only observed during plantar flexion and quantitative strain measurements were only made in the muscle mid-belly. The data obtained during this project, although from only a limited number of subjects, demonstrated a trend in the events taking place in a complex muscle group.

We originally proposed to measure 3-dimensional motion, measure strain under load bearing motion and examine an injured calf. These were not all achieved, mainly because of the following two factors:

1. A limited number of prescribed time slots were available on the clinical MRI scanner as a result of the large service provision burden. These scan time limits also did not allow for prolonged periods of experimentation while the subjects were in the scanner, thereby limiting detailed analysis to a single 2-D plane rather than a 3-D volume.
2. Funding was not available to increase the number of subjects to be imaged and construct the appropriate restraining devices needed to hold the leg in position during cyclical flexion and extension.

3. Load-bearing flexion cycles could not be performed since the custom built platform and pulley system was unable to withstand the forces generated during load bearing plantar flexion.

Dissertation outline

Part A presents the protocol initially submitted for this project.

Part B provides a background of skeletal muscle structure and function, the imaging techniques available and a description of the MRI sequences used in this project.

Part C presents a concise analysis of the project, including summarized introduction, materials and methods, results and discussion sections.

Part D comprises the ethics approval, consent forms, and journal style guidelines.

Part A: Protocol

Introduction

The objective of this study is to apply Magnetic Resonance Imaging (MRI) using displacement-encoding with stimulated echoes (DENSE) to measure and display the dynamic two-dimensional displacements and strain fields in the gastrocnemius muscle groups. The intention is to reproduce the experimental environment created by Zhong et al[1] in the biceps muscle and to apply this to the gastrocnemius muscle.

Background

Calf muscle injuries account for significant incapacity in marathon runners. Isolated muscular strains in the calf most commonly affect the medial head of the gastrocnemius muscle. The gastrocnemius muscle is at high risk for injury in part because it crosses both the knee and ankle-joints. Making an accurate diagnosis and predicting outcome by imaging should facilitate recovery. As part of this process, a better understanding of the normal dynamics of the muscle is helpful. Measuring the strain that healthy fibres undergo during normal motion provides an objective assessment of this dynamic interrogation.

MRI and musculoskeletal ultrasound are the two standard means of imaging dynamic skeletal muscle motion. MRI is typically performed using T1- and T2-weighted sequences without contrast medium, while occasionally fluid-sensitive, fat-suppressed sequences are routinely performed.

Previous studies capturing the dynamic motion of muscles with *in vivo* ciné MRI techniques have been limited to measurements in single planes. We will attempt to examine normal muscle movement in multiple planes to improve the evaluation of both normal and healing calf muscle.

MRI software

The MRI sequencing technique of "*d*isplacement *en*coding with *s*timulated *e*choes" (DENSE) has been widely used in cardiac imaging as it provides an excellent means of detailed displacement measurement of individual muscle fibres. To date there have been only two studies [1,6] done using DENSE imaging of skeletal muscle motion *in vivo*. None of these studies has specifically examined the gastrocnemius muscle and no three-dimensional model of muscle behaviour using DENSE has been created.

Method

In this pilot study, five healthy volunteers will be examined using the ciné DENSE sequence on the 1.5T Siemens Symphony MRI in the Division of Radiology, Groote Schuur Hospital, University of Cape Town.

The medial head of gastrocnemius muscle will initially be imaged in axial, sagittal and coronal planes during repetitive, regular flexion and extension within the bore of the MR.

- The ankle will be positioned in the MRI bore on a specially designed foot pedal
- Image acquisitions will be triggered at the commencement of the flexion/extension cycle
- Subjects will be scanned during passive flexion.

Specific anticipated outcomes

- Creation of a reference database of healthy skeletal muscle fibre movement of the gastrocnemius muscle using cine DENSE MR,
- Provision of further insights into skeletal muscle mechanics, including cellular level functionality,
- Creating and verifying three-dimensional skeletal muscle mathematical models and animations,
- Application of this knowledge in clinical settings while evaluating muscle injuries.

Possible publication journals:

- International Society for Magnetic Resonance in Medicine (ISMRM)
- Journal of Biomechanics
- Journal of Applied Physiology
- American Journal of Roëntgenology
- Journal of Sports Medicine

Background of researcher

Radiology registrar/resident at Groote Schuur Hospital in the Division of Radiology.

Resource requirements

- Use of the 1.5T Siemens MR scanner in Groote Schuur Hospital
- Use of the DENSE pulse sequence, currently on a research license from the

University of Virginia, USA

- Expert staff in the Department of Biomedical Engineering and Physics
- Healthy volunteers
- MRI-trained Radiographers.

Evaluation and study progress

- Weekly meetings to extract and process data with expert staff in the Biomedical Engineering Division
- Fortnightly meetings with the Radiology Divisional supervisor on progress of the pilot study

Part B: Literature review

Objectives of the literature review

Imaging dynamic skeletal muscle motion requires a comprehensive knowledge of muscle biomechanics, muscle physiology and imaging modalities. Each of these fields is in its own right a specialty and it is only through collaborative work that directed dynamic muscle imaging can be performed successfully.

The objectives of the literature review are:

- to extract key observations from these disciplines,
- search for similar attempts at strain-imaging not necessarily related to skeletal muscle,
- to establish the extent that medical imaging modalities have been used in dynamic skeletal muscle motion evaluation, specifically in complex units such as the calf or thigh.

Methods from mechanical engineering (e.g. strain evaluation), computer science (e.g. numerical methods), and clinical neurophysiology are commonly combined for the analysis of muscle biomechanics. The specific biomechanical metric used in this study is strain, which is loosely defined as the ratio of the measurements of deformed state of a material to its initial undeformed state. Techniques for non-invasively measuring strain *in-vivo* have been developed using ultrasound and MR imaging modalities. The literature review includes an assessment of these techniques, as well as the algorithms employed for processing the imaging data to derive the strain measurements. The most appropriate or commonly accepted tool required for this analysis can then be selected based on these findings. Of note, the term "strain" as applied to muscle injury is differs from the engineering 'strain' that one measures under experimental conditions. A 'muscle strain' is a loosely applied term used in clinical practice to describe an injured muscle. 'Mechanical strain' refers to a deformation that a muscle fibre undergoes which can be quantified mathematically.

MRI typically provides images of stationary anatomy (other than in cardiac imaging, which forms a very small proportion of the total MRI examinations). Applying dynamic imaging requires an understanding of the limitations of MR imaging and the strategies available to cope with motion while the imaged object is in the MRI scanner

Search strategy

Keywords: calf muscle, cine MRI, DENSE, strain, skeletal muscle, muscle imaging, muscle physiology

- Structured literature search on the following platforms was performed: Pubmed, Science Direct and Google Scholar,
- Direct contact with experts in the field by making contact with authors from relevant journal publications,
- Interviews with specialist staff at the Sports Science Institute, Cape Town

Inclusion criteria

- All references from established radiology journals using MRI or ultrasound to examine skeletal muscle or cardiac muscle,
- Articles from journals on biomechanics, bioengineering and physiology and similar recognized, peer-reviewed journals.
- Relevant congress abstracts that may not have yet been published.

Exclusion criteria

- Journals, books and links not on the above mentioned search platforms.

Summary of interpretation

The objective of this study was to apply ciné Magnetic Resonance Imaging (MRI) using Displacement-Encoding-with-Stimulated-Echoes (DENSE) to investigate the dynamic displacements and strain fields in the medial belly of the gastrocnemius muscle in healthy subjects. The primary outcome was to assess displacement and strain patterns in normal healthy muscle during motion in a complex muscle group.

In pennate muscles, fascicles are arranged obliquely with respect to the tendon, and this angulation (pennation angle) changes during contraction [2]. The forces exerted by muscle fibers are therefore modified at the fascicle level to characterize the force-generating capabilities of a muscle. Pennate muscles also have long tendons and aponeuroses with substantial compliance which modulate the force-generating capabilities of a muscle by causing changes in fascicle length as force is exerted at a given joint angle [2]. These factors make it difficult to estimate muscle actions solely from observation of joint motion. Attempts have been made to determine the geometric arrangement of muscle fibers or fascicles (muscle architecture) in humans, including measurements of cadaver specimens [2]. It has been shown in both animals and humans that muscle architecture changes by contraction even in isometric actions. Deductions on human muscle architecture based on human cadaver specimens might therefore not accurately represent the profile of actively contracting muscle bundles. Consequently, there are particular advantages to using noninvasive techniques to determine the muscle architecture in living subjects.

Calf-muscle injuries cause significant incapacity in marathon runners [3]. In particular, calf strains are generally regarded as common injuries, particularly in track and field athletes, although specific data on injury rates are sparse [4]. The ‘‘calf muscle’’ or triceps surae consists of three separate muscles (the gastrocnemius, soleus, and plantaris) whose aponeuroses unite to form the Achilles tendon. Localization of the injured muscle is routinely undertaken by using combinations of clinical history, physical exam, and imaging studies. Differentiating strains in the gastrocnemius and soleus is particularly important for accurate prognosis, appropriate treatment, and successful prevention of recurrent injury.

The gastrocnemius muscle is at high risk for injury, in part because it crosses both the knee and ankle-joints, and also as it has a high proportion of type2 fast-twitch fibres [3]. Isolated muscular strains in the calf most commonly affect the medial head of the gastrocnemius muscle [5]. The combination of biarthrodial architecture leading to excessive stretch and rapid forceful contraction of type-2 muscle fibers is thought to result in strain. In the MRI study of strains and tears undertaken by Boutin *et al*. [2], the medial head was more frequently (86%) involved than the lateral head, and low-grade or partial tears were more common than complete tears.

Information on muscle architecture related to joint position is essential for the study of muscle function [2]. The triceps surae muscles are the main synergists for plantar flexion. The gastrocnemius muscles are two-joint muscles crossing both the knee and ankle joints, whereas the soleus is a single joint plantar flexor. Consequently, the relationships among joint angles (of both knee and ankle joints), muscle (and therefore fascicle) lengths, and pennation angles are highly specific to individual muscles.

Skeletal muscle has a complex dynamic structure in which thousands of force-producing muscle fibres are arranged within a connective tissue network. The cooperative interactive behaviour of muscle fibres arranged within a muscle group is not well understood. Muscle can be represented as an extensive 3D set of endomysial tunnels within which the myofibres operate [6]. Myofibres are known to be connected to the extracellular matrix and neighbouring muscles [6], The multimolecular complexes connecting sarcomeres to elements of the subsarcolemmal cytoskeleton, and from there to trans-sarcolemmal molecules, provide a route of force transmission to the extracellular matrix [6]. This knowledge has not generally been used explicitly in physiological muscle experiments evaluating the role that these connections may play in force transmission. There is evidence to suggest that lateral tension between fibres plays a significant role in force generation and transmission. Street *et al.* have demonstrated and quantified the contribution to muscle contraction made by this lateral transmission of forces through the microfibrillar network ("transverse coupling"), as well as between the myofibrillar components and sarcolemma

[7]. They further established that both the lateral and longitudinal transmission of tension is approximately equal. Both active and resting tension was seen to be transmitted laterally. Yucesoy *et al.* divide skeletal muscle motion into (1) the intracellular and (2) extracellular matrix domains [6]. They suggest that evaluation of these mechanics should include assessment of both the trans-sarcolemmal attachments of the muscle fibre's cytoskeleton, as well as the extracellular matrix. Sampath *et al.* found a larger-than-expected length range for force production in both human rectus femoris and rat semimembranosus muscles [8]. They suggest that it is the heterogeneity of the mean sarcomere length within the muscle belly that permits broadening of the force-length curve, enhancing the range of active force generation.

Most models represent muscle properties using simple geometric idealizations that assume that all muscle fibers shorten uniformly [1]. These models are limited in their ability to accurately represent the *in vivo* behavior of muscles that have complex arrangements of muscle fibers or constitute part of a complex group of muscles such as the calf muscles.

Several mathematical models aid in the study of muscle mechanics. These include the finite-element analysis method, the lumped-parameter model and line-path-segment representations, using point definitions and surface wrapping [10]. These models have been experimentally tested *in vivo* and used to extrapolate data to better understand muscle mechanics.

Recently, several investigators [6, 9] have developed finite-element models of skeletal muscle that allow for the representation of realistic 3D geometries, incorporating the nonlinear active and passive constitutive properties of muscle tissue, and are able to characterize non-uniform shortening within muscles These models have provided new insights into skeletal muscle mechanics; for example, analysis of a finite-element model of the biceps brachii muscle identified the complex features of muscle architecture that could contribute to non-uniform strains along muscle fascicles [1].

Three-dimensional high-resolution imaging should improve global understanding of muscle mechanics as this acquires information about the complex motion path of muscle fibre groups, as well as events in the extracellular milieu without the need to manually define regions of interest (ROI’s).

Ultrasound and MRI are the two standard means of imaging skeletal muscle. MRI is typically performed using T1- and T2-weighted sequences without intravenous contrast medium [11], while occasionally fluid-sensitive fat-suppressed sequences are also employed [3]. The examinations are performed in at least two orthogonal planes. In addition to the requisite axial plane, a second long-axis plane is generally acquired sagittally (when evaluating abnormalities at the anterior or posterior aspect of an extremity) or coronally (when evaluating abnormalities at the medial or lateral aspect of an extremity). Either modality is useful in the initial diagnosis of acute injury or sprain.

Ultrasound may be particularly useful when used as part of the initial clinical examination by the sports medicine physician, when severe pain and swelling limit clinical testing. It may also be valuable in early triage of calf injuries or complaints when a wider differential is considered. It has advantages of cost, portability, speed, and ease of use, compared to MRI when in the hands of an experienced operator. Ultrasonography can provide images of length changes, fibre pennation and gross muscle motion of skeletal muscle fascicles [12]. Knowledge of this *in-vivo* architecture provides important information that enables comparative analysis with data obtained at MR.

A limited number of studies have reported strain imaging using ultrasound on skeletal muscles [12] . One of the first *in-vivo* efforts was performed with Doppler-based techniques [13]. Ultrasound “elastography” was introduced by Ophir et al [13] as a technique to estimate the mechanical properties of biological tissues and organs from the relative deformation, i.e. , strain. Local displacement was assessed by correlating segments of ultrasound data acquired sequentially. By calculating the first-order spatial derivative of the displacement field(s), “strain images” were obtained. Initially, the technique was used to visualize the distribution of strain within tissue under external compression i.e., a passive deformation. More recently, strain imaging has also been applied to data acquired in actively deforming tissue, such as the heart, to assess its function [13].

Recent work by Galban *et al.* using MRI-based diffusion tensor imaging (DTI) demonstrated the ability of DTI to differentiate between functionally different muscles in the same group of muscles, based on their diffusion properties [14]. The theoretical basis of DTI is that cell membranes and other structures constrain water diffusion in tissues leading to anisotropic diffusion. Water movement can be evaluated by determining the three orthogonal directions of water diffusion, called eigenvectors, and their intensities, called eigenvalues. From the three eigenvalues ($\lambda 1$, $\lambda 2$ and $\lambda 3$), parameters such as fractional anisotropy (FA) or apparent diffusion coefficients (ADC) can be calculated to evaluate the character of water diffusion in a voxel. DTI probes muscle tissue at a microscopic scale and can provide information about the overall structure of skeletal muscle. Many studies have demonstrated that anatomically the first eigenvalue represents the diffusion along the main direction of fiber muscle [15, 16]. In addition, it has been suggested that the second and the third eigenvalue (diffusion perpendicular to the fiber direction) could represent the diffusion of water within the endomysium and throughout the fiber radius, respectively. Other anatomical features of muscle obtained with DTI include cross-sectional area, fiber length and the pennation angles of muscle fibres.

Cine MRI is able to produce a dynamic series of anatomical images of a structure when it is undergoing cyclical motion. The cine MR sequence, steady state free precession (SSFP) that is commonly used for cardiac imaging, is a robust, rapid acquisition sequence that can grossly delineate skeletal muscle anatomy and accurately demonstrate macroscopic dynamics [17]. This allows for relatively good signal-to-noise and contrast-to-noise ratios, and can be acquired within a relatively short acquisition time.

Standard cine MRI sequences require multiple cycles of gated motion; typically 60–120 repetitions are needed to acquire composite images representing one motion cycle. This requirement of many motion cycles creates several problems. The quality of the images degrades dramatically if the motion cycles are not repeated accurately, only low numbers of cycles can be studied due to fatigue and subjects with musculoskeletal or neurologic diseases who cannot complete a large number of repeated motions cannot be studied with cine MRI techniques. This limits the use of cine MRI for musculoskeletal applications.

Real-time MRI is a newer method of skeletal muscle analysis [19]. This technique can image the anatomy and muscle tissue velocities in a single cycle of motion and it may offer improvements over cine MRI for the study of muscle function

Cine phase-contrast MRI (CPC-MRI) has been widely used to investigate the mechanics of musculotendinous structures and basic muscle contraction mechanics. It is a combination of the cine-MRI and phase contrast MRI techniques that can provide anatomical information and the kinematics of an object performing a cyclic motion [18-20]. To apply CPC-MRI for general skeletal muscle modeling, two problems need to be solved: the distribution of continuous regions of interest (ROIs) and a general description for possible large and complex deformation patterns. Proposed solutions include measuring the relative distance change between ROI's and using engineering strain to define normalized deformation [19].

Biomechanical models generally assume that muscle fascicles shorten uniformly [7]. However, dynamic MR images of the biceps brachii have recently shown non-uniform shortening along some muscle fascicles during low-load elbow flexion [21]. Factors contributing to this are thought to be the variation in fascicle length [2], the curvature of the fascicles and the mechanical heterogeneity of muscle and tendon. Uniform shortening of muscle fibres may not exist in biological reality, as the whole muscle is a complex composite of contractile and connective tissue elements. For example, CPC-MRI images taken of the long head of the biceps brachii showed non-uniform shortening along some muscle fascicles during low-load elbow flexion [21]. In that study, the displacements of square regions of interest were calculated by integrating the velocity measurements, while one-dimensional strains were determined by calculating the change in length between square regions that were placed along the muscle fascicles. These data provide valuable in-vivo measurements to confirm the models' predictions of non-uniform strains along fascicles.

However, in addition to non-uniform shortening along muscle fascicles, finite-element models also predict non-uniform strains transverse to the fascicle direction [9]. Another method for directly measuring displacement is fibre-tagging [22, 23]. Limited work has been done using this technique for skeletal muscle motion analysis - to our knowledge, the only formal studies focused on motion of the human tongue [24]. The main disadvantages of this technique are reduced spatial resolution and the need for time-consuming manual intervention and selection in data-analysis

*D*isplacement *en*coding with *s*timulated *e*choes (DENSE) is an MRI technique for measuring tissue displacement [25]. In DENSE, tissue displacement is encoded into the phase of the stimulated echo resulting in a pixel-wise map of displacement, which can in turn be used to derive measures of regional deformation or strain. In ciné DENSE, a time series of displacement maps [26, 27] is derived, allowing for a dynamic analysis of tissue displacement and strain. The DENSE technique has been widely used in cardiac imaging [25, 26, 28] as it provides an excellent means of detailed displacement measurement of individual muscle fibre groups [25].

While phase-contrast velocity encoding measures instantaneous velocity, DENSE measures displacement in relation to encoding time. The DENSE method uses stimulated echoes to provide high spatial-density measurement of displacement. MR imaging with DENSE offers many of the advantages of both fibre-tagging and velocity-encoded imaging. As with tagging, DENSE MR imaging involves spatial modulation of magnetization to position-encode the magnetization at one point in a motion cycle and to subsequently image tissue displacement relative to this starting point later in motion cycle [26]. Similar to velocity-encoded images, phase-reconstructed images are obtained by DENSE MR imaging in order to achieve pixel-wise spatial resolution and thereby direct extraction of motion data (in this case, displacement rather than velocity).

DENSE offers a robust method for quantifying 2-D strain fields [25, 26]. Using a motion phantom, cine DENSE has previously been shown to be highly accurate [27]. To date, there have been only three studies using DENSE imaging of skeletal muscle motion *in vivo* [1, 29, 30]. Zhong et al [1] were able to determine the one-dimensional linear strain distributions extracted from the cine DENSE measurements were consistent with previous cine-PC measurements in the same muscle (biceps brachii) [21]. They demonstrated that

2D strains were non-uniform throughout the biceps brachii muscle during low-load elbow flexion and that the directions and magnitudes of the first and the second principal Lagrangian strains were non-uniform throughout the muscle. Zhong et al [1] showed how the strain data from their MR findings differed from the muscle architecture determined by ultrasound. The muscles fascicles in the region of interest were oriented at an acute angle to the midline fascial planes. Their strain results suggested that the principal direction of shortening varied throughout the muscle and was not necessarily aligned with the muscle fascicle direction. Fiorentino et al [29] utilized a three-dimensional (3-D) cine DENSE imaging sequence over a volume of tissue in the thigh during knee joint flexion. They analyzed the 3D displacements of the quadriceps and hamstring muscles and were able to demonstrate the feasibility of DENSE in acquiring 3-dimensional data. To our knowledge this is the first cine DENSE work that specifically examines the gastrocnemius muscle.

The potential clinical application of this type of strain imaging would primarily be directed at athletes undergoing rehabilitation after sustaining a muscle injury. Rehabilitation programmes often involve strength training of adjacent muscle groups to maintain the athlete’s fitness and compensate for the injury while repair takes place.

While a healing tear can confidently be evaluated by serial MRI examinations [31], little additional information concerning molecular/microstructural events in neighbouring muscles is gained. Advanced imaging strategies may be usefully directed towards the assessment of the degree of strain [32] occurring in these ‘normal’ compensating muscles, in order to prevent inadvertent overcompensation and additional injury of these muscles during rehabilitation. For example, Okamoto *et al.* were able to relate minor muscle damage occurring in adjacent muscles after load-bearing in the lower limb to fractional anisotropy as derived using the DTI technique [33].

Further research application

Cine DENSE imaging has the potential to provide the data needed to improve the understanding of muscle contraction mechanics, and to evaluate predictions made by muscle models. Muscle pathologies are often manifested by alterations in fibers and connective tissue [1].Analyzing skeletal muscle mechanics using cine DENSE imaging in persons with muscle pathology may lead to an advanced understanding of the manner in which these alterations affect muscle behavior and function. These results, combined with computational models of muscle, may lead to more accurate and individualized muscle models that can capture effects of pathology and be used to gain new insights into the causes of movement abnormalities and to simulate novel treatment strategies [1].

A reference range of normal strain values for non-injured calf muscle is necessary to provide comparative data for strain imaging on injured muscles. The aim would be in part to evaluate the injured muscle, but also to interrogate events in neighbouring muscles during the rehabilitation process, thereby preventing inadvertent secondary injuries. Strain imaging may also in future represent a method for objectively determining whether a sports injury has completely healed.

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Part C: Results

Cover letter

All authors have made substantial contributions to all of the following:

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data,

(2) drafting the article or revising it critically for important intellectual content,

(3) final approval of the version to be submitted.

Acknowledgements: no outside acknowledgements.

No writing assistance, apart from draft manuscript reviews by the co-authors listed above, was used.

The manuscript, including related data, figures and tables has not been previously published and is not under consideration elsewhere.

This manuscript has been submitted as a short communication to the Journal of Biomechanics, where word count may not exceed 1500 words.

Conflict of interest statement

None of the authors has any financial or personal relationships with other people or organisations that could inappropriately influence (bias) their work.

Referee suggestions

None

Title page and abstract

Imaging displacement and strain in the medial gastrocnemius muscle during ankle-joint motion using 2D ciné DENSE MRI

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Keywords:

Strain, medial gastrocnemius, skeletal muscle, ciné DENSE MRI

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Abstract

PURPOSE

The objective of this study was to apply Magnetic Resonance Imaging (MRI) using Displacement Encoding with Stimulated Echoes (DENSE) to investigate the dynamic displacements and strain fields in the medial gastrocnemius muscle.

METHOD AND MATERIALS

Five healthy volunteers were imaged using a 1.5T Siemens MRI scanner (MAGNETOM Symphony, Siemens, Erlangen). The study was approved by our institutional review board (Rec ref 383/2009).

After informed consent was obtained, the volunteers were positioned feet-first and right-side down into the tunnel of the MRI scanner. Their right ankles were positioned on a platform, with a fully extended right knee. A flexi-coil was wrapped and secured around the right calf. The sequence was gated using a plantar-flexion trigger mechanism, and a series of ciné echo-planar DENSE images was acquired over several successive plantar flexions. Displacement encoding was applied in three orthogonal directions, using the following imaging parameters: field of view= 245 × 357 mm^2, slice thickness = 8 mm, flip angle = 15 degrees, TR = 10 ms, TE = 4.8 ms, displacement encoding frequency = 0.05 cycles/mm. The belly of the medial gastrocnemius was manually defined on the DENSE magnitude images. First principle-strain measurements (reflecting maximum muscle shortening) were derived from the displacement fields after spatio-temporal phase unwrapping, and the strain values were normalized to muscle length.

RESULTS

Regional displacements were largest in the medial portion of the medial gastrocnemius, adjacent to the interface with the soleus muscle. The directions of the first principle strain vectors agreed well with the pennation angle of the myofibres. The magnitude of the first principle strain was largest in the middle of the muscle belly. The mean first principle strain along this midline at maximum plantar flexion for all five volunteers was 60.2±32.5%.

CONCLUSION

This study shows how ciné DENSE MRI can be used to quantitatively determine regional displacement and strain in the gastrocnemius muscle. The technique provides insight into the kinetics and deformation of an individual muscle component/belly moving within a complex muscle group. Understanding healthy strain patterns in skeletal muscle may assist in monitoring the recovery of injured muscle.

Manuscript

Introduction

The objective of this study was to apply ciné magnetic resonance imaging (MRI) using displacement encoding with stimulated echoes (DENSE) to investigate the dynamic displacements and strain fields in the medial belly of the gastrocnemius muscle in healthy subjects. The primary outcome was to assess displacement and strain patterns in normal healthy muscle during motion in a complex muscle group.

Skeletal muscle has a complex dynamic structure in which thousands of force-producing muscle fibres are arranged within a connective tissue network. Dynamic MR images of the biceps brachii have shown non-uniform shortening along some muscle fascicles during low-load elbow flexion [1]. Uniform shortening of muscle fibres may not exist in biological reality, as the whole muscle is a complex composite of contractile and connective tissue elements. Factors that may contribute to this include the variation in fascicle length [2], the curvature of the fascicles, and the mechanical heterogeneity of muscle and tendon.

Displacement encoding with stimulated echoes (DENSE) is an MRI technique for measuring tissue displacement [3]. It is predominantly used in cardiac imaging to produce pixel-wise maps of tissue displacement and strain. In ciné DENSE, a time series of displacement maps is derived, allowing for a dynamic analysis of tissue displacement [4].

Previous application of ciné DENSE in skeletal muscle imaging focused on a 2-dimensional interrogation of the biceps brachii muscle [5] and a 3-dimensional examination of the quadriceps group [6]. Zhong et al. [5] suggests that the principal direction of shortening varies throughout the muscle and is not necessarily aligned with the muscle fascicle direction, and Fiorentino et al. [6] conclude that each individual muscle moves in a complex non-planar fashion within muscle groups. These studies both involved relatively isolated moving muscles.

This study uses ciné DENSE to interrogate gastrocnemius muscle strain patterns during plantar flexion. We hypothesize that the spatial strain distribution will again be non-uniform, and that significant interfascicular shearing will occur during motion because the gastrocnemius forms a part of a complex interdependent muscle group.

Materials and Methods

Experimental Setup

Five healthy volunteers (3 males and 2 females, average age 21.2 yrs, range 19-25 yrs, average weight 70.6 kg, range 60-95 kg) were imaged using a 1.5T Siemens MRI scanner (MAGNETOM Symphony, Siemens, Erlangen). Informed consent was obtained and the study was approved by our Institutional Review Board.

As portrayed in Figure 1a, the volunteers were positioned feet-first and right-side down, lying parallel to the bore of the MRI scanner. Their right ankle rested on a platform and the right knee was held fully extended. The subject's right thigh and ankle were secured to the table with Velcro straps to ensure that the leg remained stationary during calf motion. A flexi-coil was wrapped around the right calf and secured to a frame designed to avoid contact between the moving calf belly and the coil. The ciné image acquisition was gated using a triggering device which was secured to the foot platform and designed to activate when the dorsal surface of the forefoot made contact with the trigger lever (Figure 1b). Subjects were instructed to start motion from full dorsiflexion, and then perform repetitive cycles of full plantarflexion to full dorsiflexion over an approximate 45 degree arc at a rate of 30 cycles per minute (i.e. 2 seconds between two neighbouring trigger activations). The rate was maintained with reference to a metronome tone played through the MRI headphones.

Image acquisition

Localisation was performed using a stack of T1-weighted axial images with the foot in a relaxed, neutral position (TR = 700 ms, TE = 24 ms, FOV = 245×357 mm^2, image matrix = 256×128 voxels and slice thickness = 10 mm). Using these images, an oblique sagittal plane was selected to bisect the longitudinal axis of the medial gastrocnemius and include the soleus and anterior extensor muscles (Figure 2). Axial and oblique ciné steady-state free precession (SSFP; TR = 42ms, TE = 1.3ms, FOV = 250x330 mm^2, image matrix = 192x94) images were obtained during repetitive motion to ensure that through plane motion was minimized in the selected oblique sagittal imaging plane. All ciné imaging was gated over several successive plantar flexion/extension cycles.

A ciné series of echo-planar DENSE images was then acquired in both axial and oblique sagittal planes. Displacement-encoding was applied in three orthogonal directions, using the following imaging parameters: TR = 9.7 ms, TE = 4.6 ms, FOV = 360×240 mm^2, image matrix = 128 × 88, displacement encoding frequency = 0.05 cycle/mm, and temporal resolution = 30 ms, non variable flip angle of 15 degrees.

Dynamic ciné ultrasound using a high resolution linear probe (Philips IU22) was also performed on one of the volunteers.

Data analysis

Displacement and strain were derived offline using custom tools written in MATLAB (The Mathworks Inc., Natick, MA. United States). Phase-unwrapping, tissue tracking, and 2D Lagrangian strain computation were performed [7], and first principal strain measurements were made along a manually defined line along middle of the medial gastrocnemius muscle belly. Strain values were normalized to knee-to-ankle length.

Results

Example DENSE phase maps at maximum plantar flexion are shown in Figure 3, where it is evident that the majority of motion occurs in the head-foot direction. Figure 3c confirms that through-plane motion can largely be ignored. Figure 4a shows a DENSE magnitude image with the region of interest drawn over the medial head of gastrocnemius, and Figure 4b and 4c show the corresponding muscle fascicle displacements and first principal Lagrangian strain at maximal flexion, respectively.

Regional displacements are largest in the medial portion of the medial gastrocnemius, adjacent to the interface with the soleus muscle, and the magnitude of the first-principal strain is largest in the midline of the muscle belly. Figure 5 shows a series of ciné frames during the flexion cycle where the first principal Lagrangian strain maps are matched to equivalent time points from the ultrasound acquisition. Table 1 shows the normalised first principal Lagrangian strain along the midline of the muscle belly of the five volunteers.

Discussion

Ciné ultrasound reveals that the soleus and medial gastrocnemius muscles move in temporal synchrony during the initiation of plantar flexion from a fully dorsiflexed starting position. In mid flexion, the underlying soleus moves faster than the gastrocnemius muscle for a short period. Following this, the gastrocnemius again becomes recruited for the mid to extreme range of plantar flexion. The deep gastrocnemius fibres displace more than the superficial fibres.

In the midpoint of the cycle, deformation of gastrocnemius muscle fibres in the mid regions of the belly becomes apparent as more fibres are recruited to facilitate movement during the remainder of the cycle. The variation in first principal strain during flexion is not consistent with the relatively constant muscle fascicular orientation seen on ultrasound. This has previously been attributed to shearing between fibres [8], a factor which is evident in the displacement gradient in Figure 4b. The gastrocnemius mid-belly fibres exhibit the greatest deformation as one would expect from a muscle acting independently. However, when examining dynamic muscle motion within a complex unit such as the calf, several other factors may play a role in the biomechanics that eventually lead to joint motion. For the medial gastrocnemius, the complex deformation and displacement characteristics that the fascicles are subjected to by adjacent muscle groups should be taken into account.

The soleus is a major contributor to ankle motion during ankle flexion, so during its passive range of motion the fibres adjacent to the soleal-gastrocnemius interface are displaced to a greater extent by the mere virtue of the bulk motion. At this stage the gastrocnemius has not been recruited in full, so only a small portion of the belly exhibits an appreciable strain.

The one-dimensional linear strain distributions in Table 1 are of a similar magnitude to those obtained from previous ultrasound studies. Hoang et al [8] estimated the mid-belly medial gastrocnemius strain to be 86.0 ± 26.8 %, which is of a higher magnitude and lower standard deviation than the 60.2 ± 32.5 % reported here. This may in part be because the range of flexion was limited to an arc of 45 degrees for our study.

Ciné DENSE imaging relies on regular, repetitive motion cycles to generate reliable data. A major limitation of this study is the dependence on the volunteers' ability to perform identical motion cycles. Modern imaging techniques using navigator projections and complex image reconstruction [9] promise to improve image quality for future self-gated ciné DENSE MRI of skeletal muscle. Volumetric ciné DENSE imaging [10] will further enhance the utility of DENSE in characterizing tissue motion in a complex muscle group such as the calf. Determining the strain patterns in normal muscles would provide a baseline understanding of molecular functionality which could be applied in future as a tool to monitor the healing process of an injured muscle during its rehabilitation. At the same time, the strain occurring in the non-injured muscles compensating for the damaged muscle, can be monitored thereby, preventing further injury.

This study shows how ciné DENSE MRI can be used to quantitatively determine regional displacements and strains within the gastrocnemius muscle fibres during ankle joint motion. The dynamic 2D displacement and strain patterns measured in this study provide insight into the kinetics and deformation of individual muscle fibre groups moving within a complex muscle group.

Figure titles

Figure 1: Experimental design. (a) Each subject was positioned lying on their right side with a general purpose flexi-coil (not shown) wrapped around the upper right calf. The connecting wire of the pedal trigger passes through the bore of the magnet and inserts into the MRI console via an electronic circuit interface. (b) Schematic representation of the trigger mechanism used to gate the ciné MRI scans.

Figure 2: (a) Static axial, T1 image of the calf showing the oblique sagittal imaging plane used for the ciné DENSE imaging. (b) Representative oblique sagittal view frame during sagittal steady-state free precession dynamic imaging with the calf at a neutral point in the cycle.

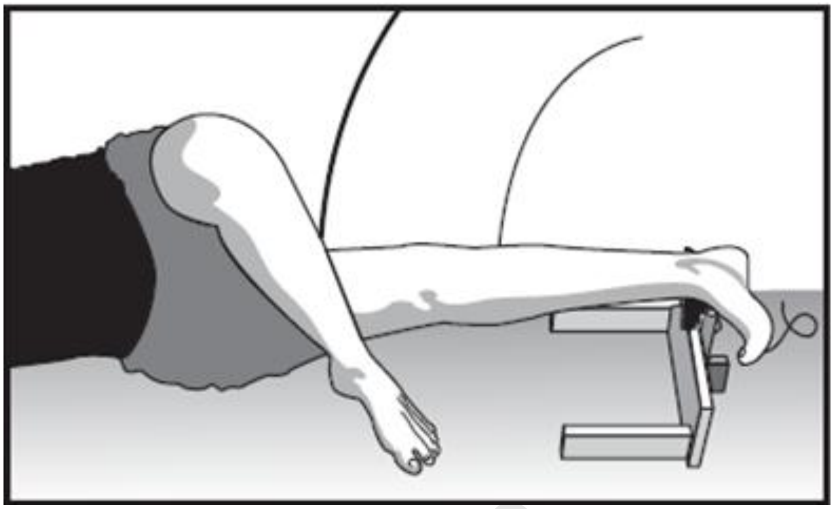
Figure 3: Example ciné DENSE phase images for (a) head-foot, (b) anterior-posterior, and (c) left-right (through-plane) directions, (d) short axis axial acquisition, (e) shows an alternative 3D representation of (d). For the phase images; dark signal indicates motion in one direction, while lighter signal represents motion in the opposite direction. The red indicates the direction of the anterior tibialis muscles while the blue indicates the opposite direction in the gastrocnemius complex.

Figure 4: DENSE magnitude image, fascicle displacement and strain analysis from one of the volunteers. (a) DENSE magnitude image outlining the medial gastrocnemius, (b) Fascicle displacement during mid plantarflexion. The largest displacements occur medially, as indicated by individual arrow lengths. (c) First principal strain magnitude and direction. Red indicates positive strain (stretch).

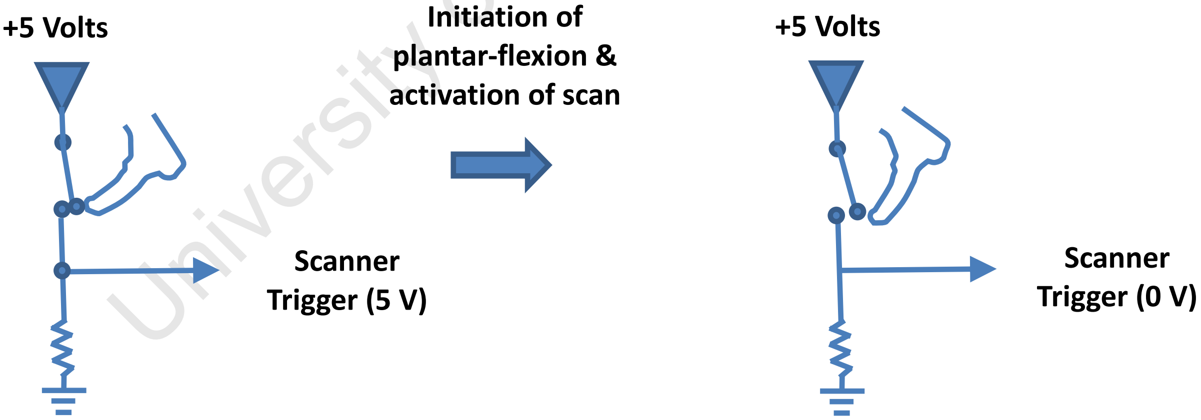
Figure 5: Series of comparable first principal Lagrangian strain maps and ultrasound images at three time points during the flexion cycle. (a) Initiation of plantar flexion, (b) early plantar flexion, and (c) mid-plantar flexion. The first principle strain increases throughout flexion. The differences between strain vector orientations and corresponding muscle fascicle directions indicate that the measured strain is not only due to muscle shortening, but also attributable to a shearing between adjacent fascicles.

Table titles

Table 1. Normalized first-principal strain values along the mid-belly of the medial gastrocnemius from 5 volunteers

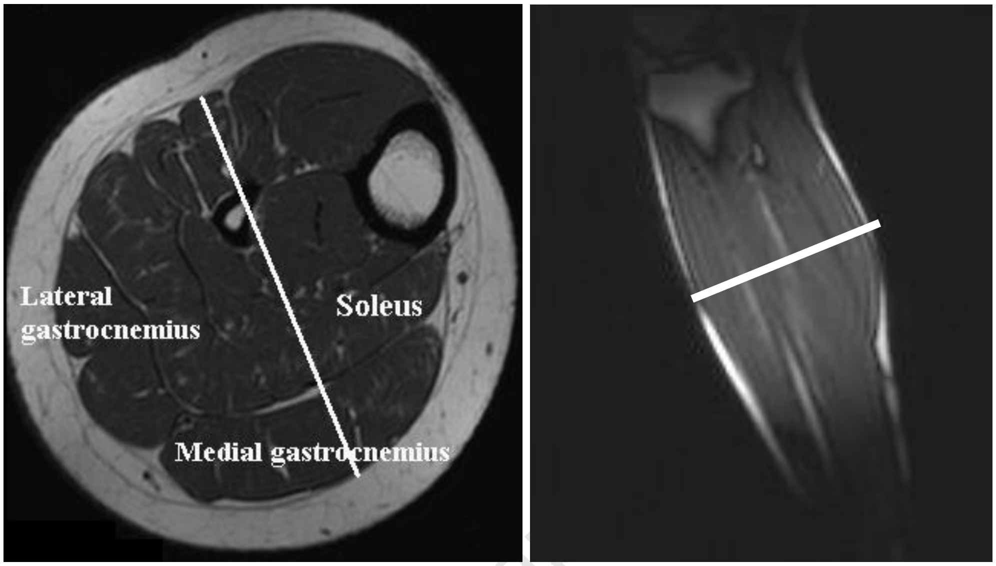


(a)


+5 Volts
Initiation of plantar-flexion & activation of scan
+5 Volts
Scanner Trigger (5 V)
Scanner Trigger (0 V)

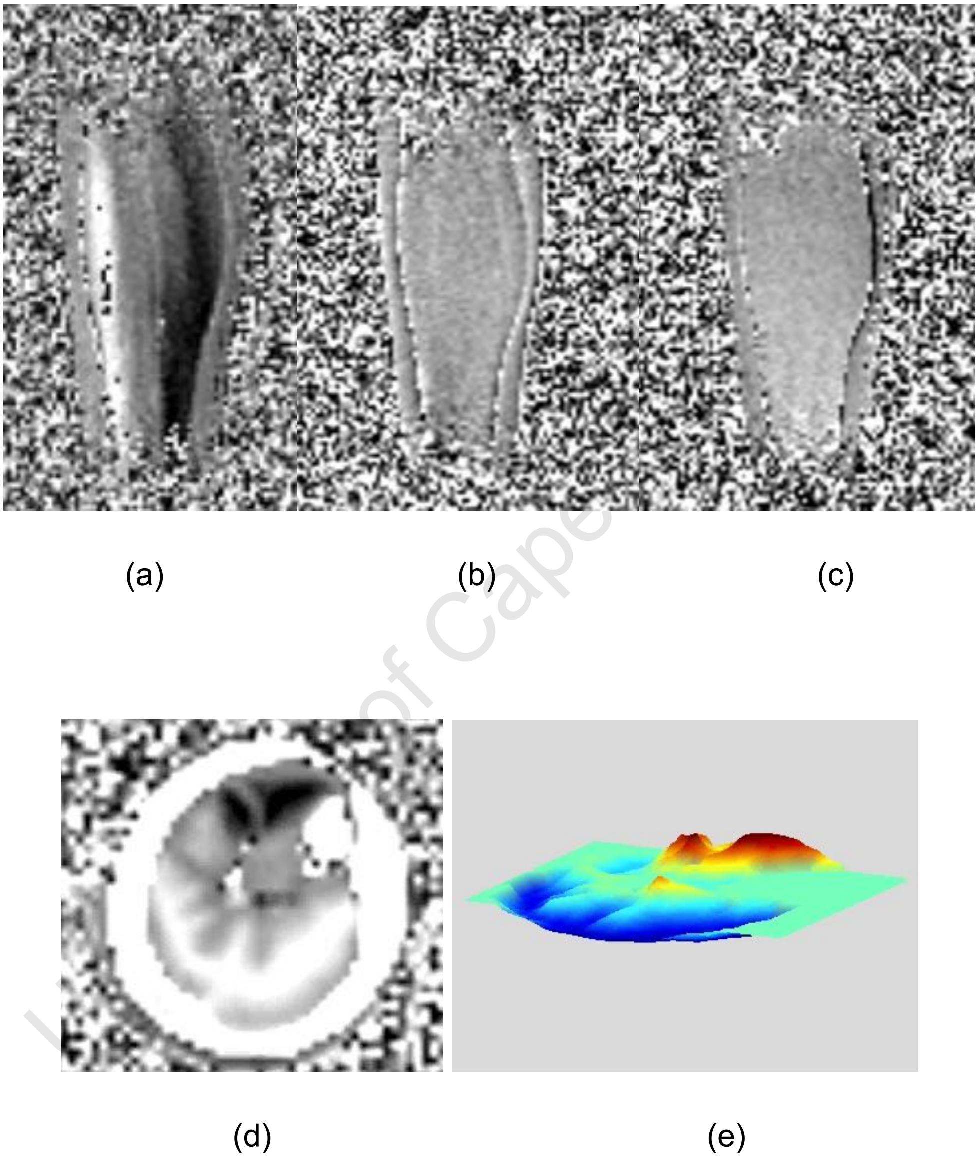
(b)

Figure 1



(a) (b)

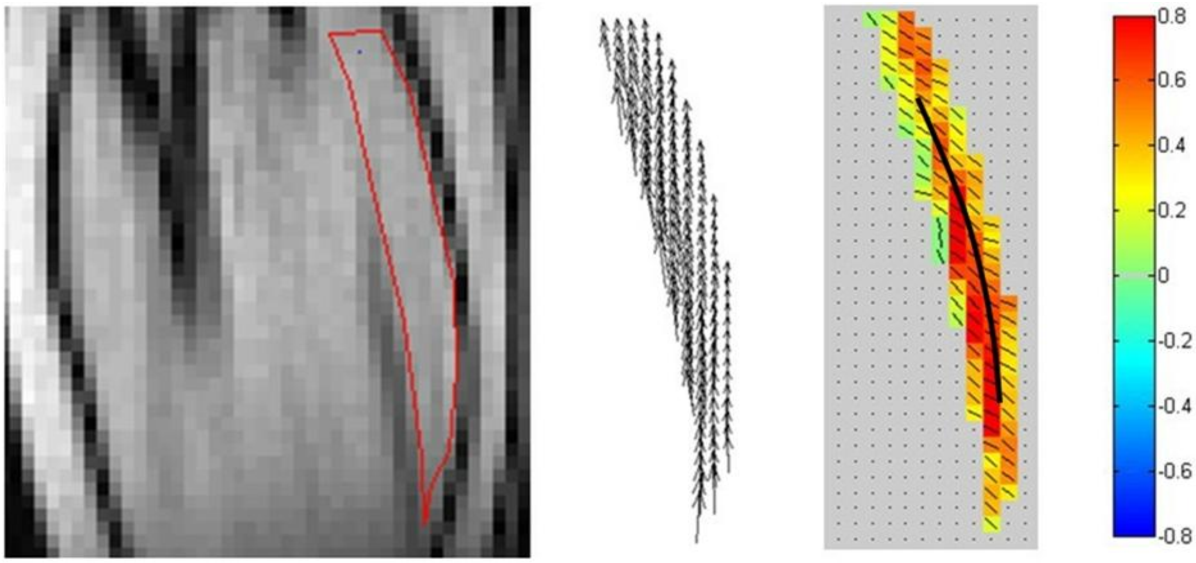
Figure 2



(a) (b) (c)

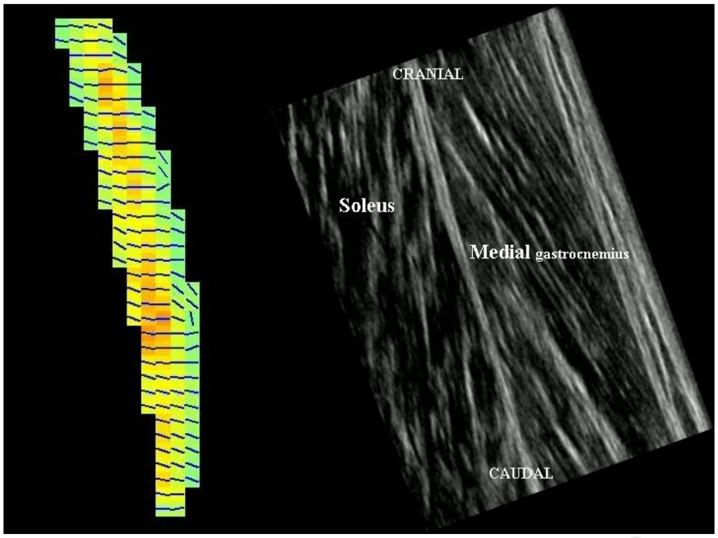
(d) (e)

Figure 3

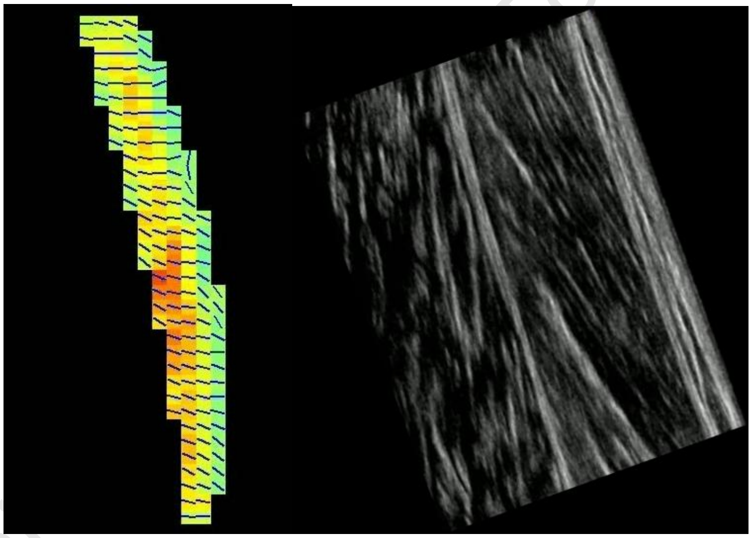


(a) (b) (c)

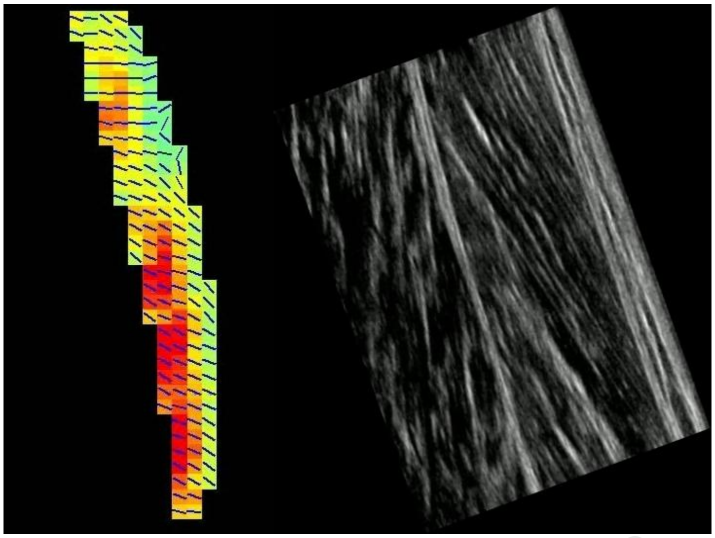
Figure 4



(a)



(b)



(c)

Figure 5

Table 1

Normalised first principal strain	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Average (%)
Mean	48.84	70.54	56.38	50.81	74.47	**60.2**
Standard deviation	18.23	51.48	33.68	20.88	38.47	**32.5**

Part D: Supporting documents

Ethics approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: nosi.tywabi@uct.ac.za

11 September 2009

REC REF: 383/2009

Dr AJ Lawson
C/o Ms Neumann
Radiology

Dear Dr Lawson

PROTOCOL TITLE: IMAGING TWO-AND-THREE-DIMENSIONAL DESPLACEMENTS AND STRAINS IN THE GASTROCNEMIUS MUSCLE DURING ANKLE-JOINT MOTION BU CINE DENSE MRI

Thank you for submitting your study to the Research Ethics Committee for review.

It is pleasures to inform you that the Ethics Committee has **formally approved** the above mentioned study.

Approval is granted for one year until 20 September 2010.

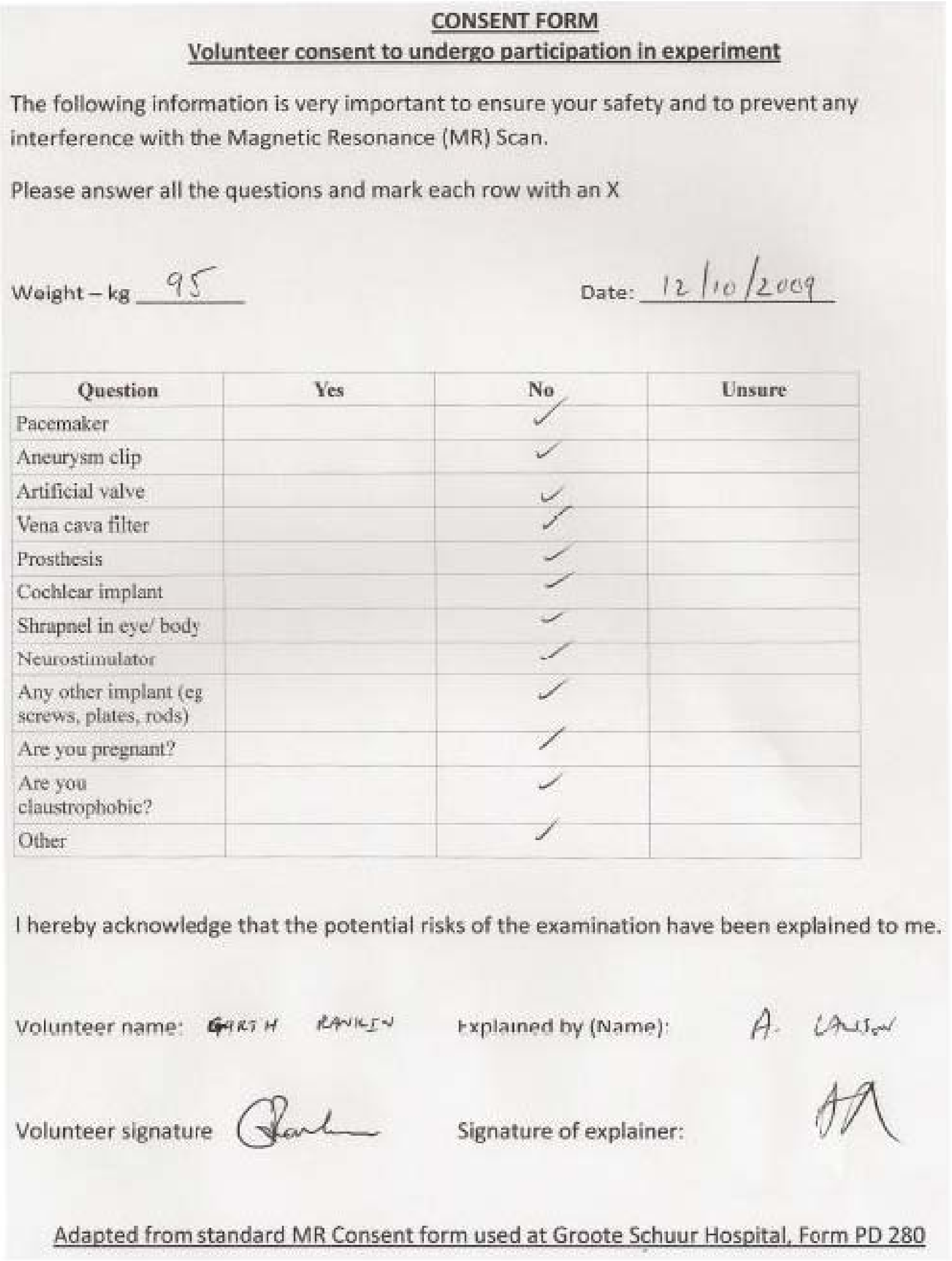
Please submit a progress report if the research extends beyond the expiry date. Alternatively, please submit a brief summary of your findings so that we can close our records.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS
This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

Consent forms



CONSENT FORM

Volunteer consent to undergo participation in experiment

The following information is very important to ensure your safety and to prevent any interference with the Magnetic Resonance (MR) Scan.

Please answer all the questions and mark each row with an X

Weight – kg 95

Date: 12/10/2009

Question	Yes	No	Unsure
Pacemaker		✓	
Aneurysm clip		✓	
Artificial valve		✓	
Vena cava filter		✓	
Prosthesis		✓	
Cochlear implant		✓	
Shrapnel in eye/ body		✓	
Neurostimulator		✓	
Any other implant (eg screws, plates, rods)		✓	
Are you pregnant?		✓	
Are you claustrophobic?		✓	
Other		✓	

I hereby acknowledge that the potential risks of the examination have been explained to me.

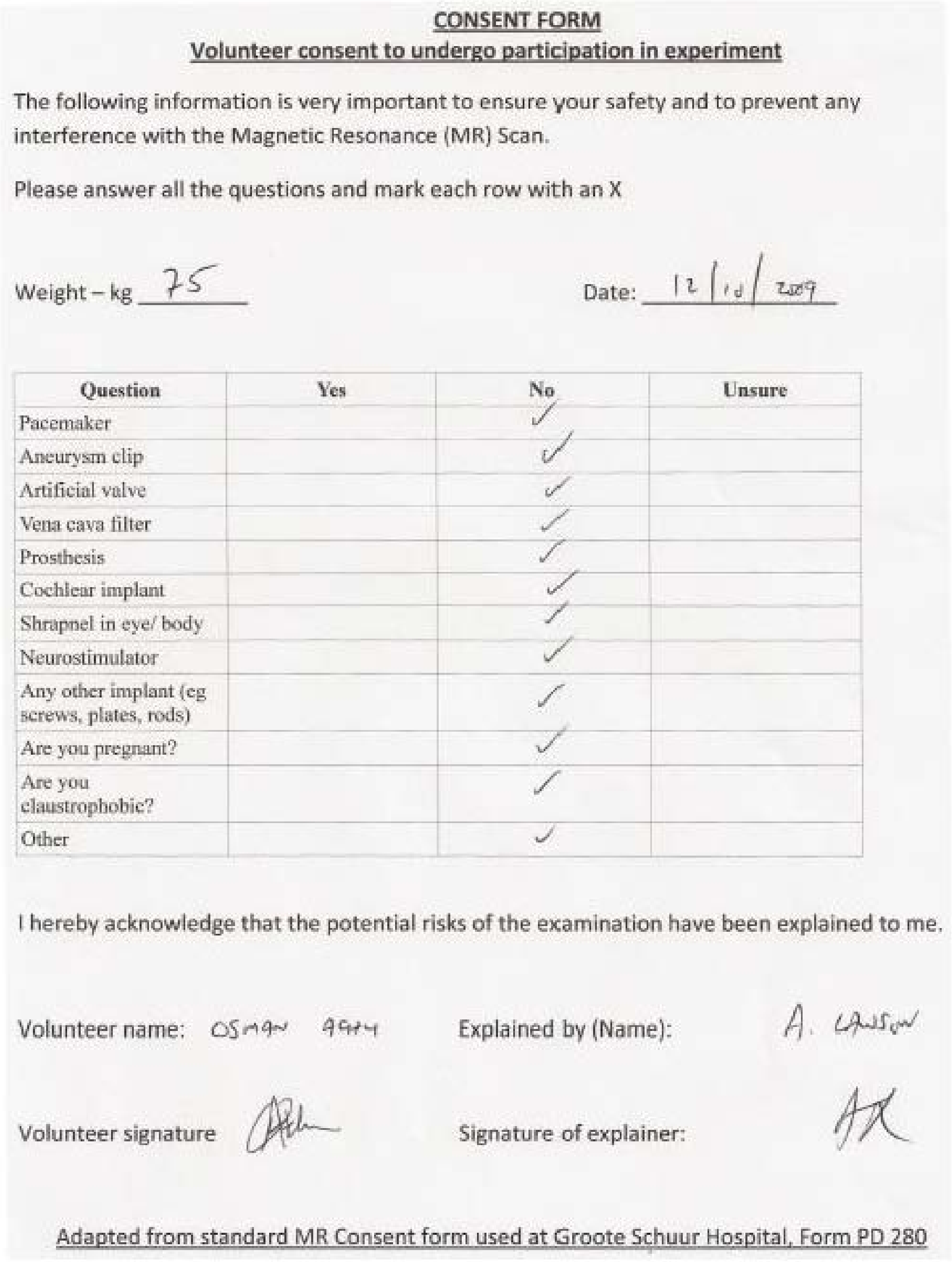
Volunteer name: GARTH RANKIN

Explained by (Name): A. LAWSON

Volunteer signature

Signature of explainer:

Adapted from standard MR Consent form used at Groote Schuur Hospital, Form PD 280



CONSENT FORM

Volunteer consent to undergo participation in experiment

The following information is very important to ensure your safety and to prevent any interference with the Magnetic Resonance (MR) Scan.

Please answer all the questions and mark each row with an X

Weight – kg 75

Date: 12/10/2009

Question	Yes	No	Unsure
Pacemaker		✓	
Aneurysm clip		✓	
Artificial valve		✓	
Vena cava filter		✓	
Prosthesis		✓	
Cochlear implant		✓	
Shrapnel in eye/ body		✓	
Neurostimulator		✓	
Any other implant (eg screws, plates, rods)		✓	
Are you pregnant?		✓	
Are you claustrophobic?		✓	
Other		✓	

I hereby acknowledge that the potential risks of the examination have been explained to me.

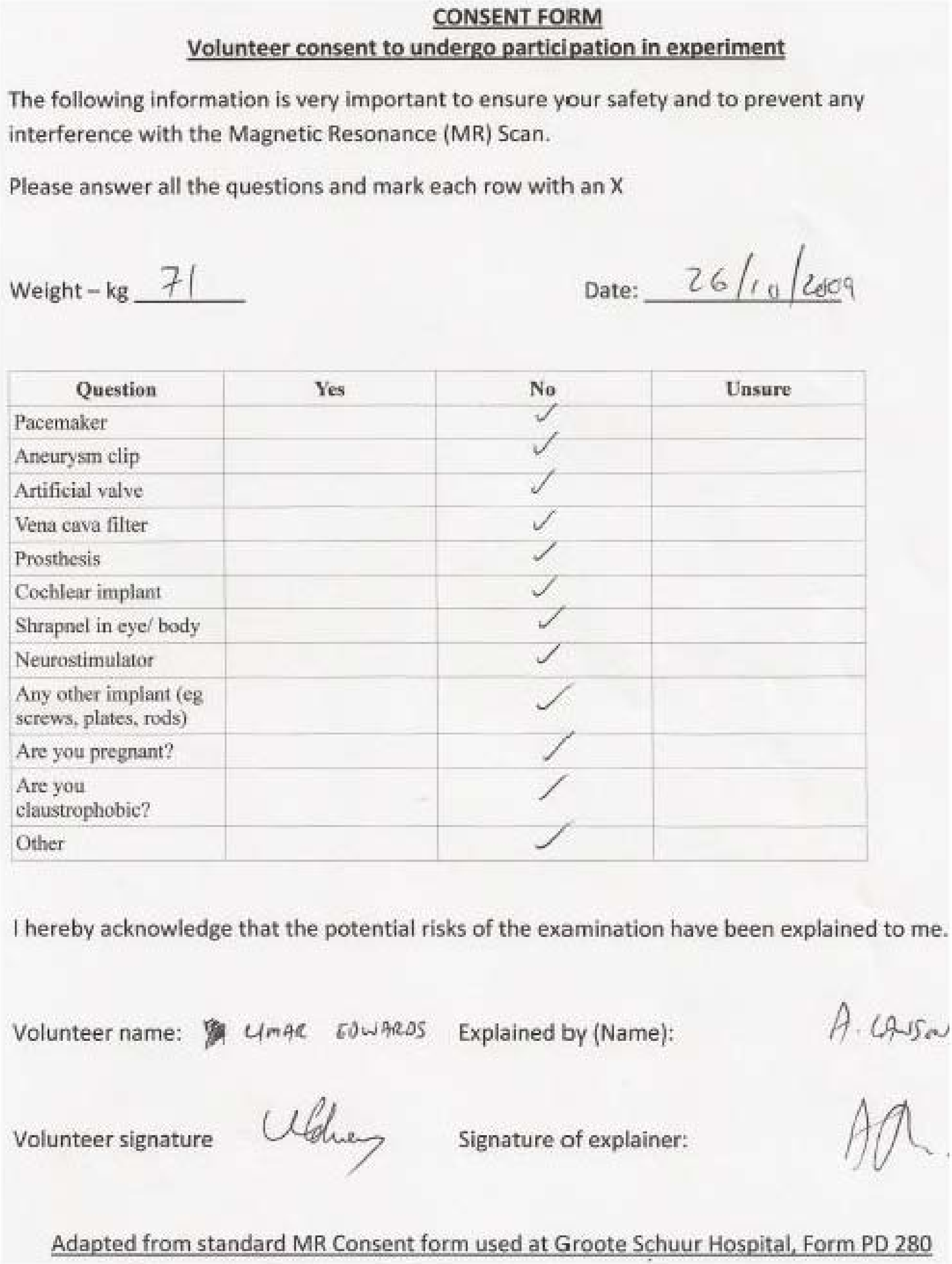
Volunteer name: OSMAN [illegible]

Explained by (Name): A. LAWSON

Volunteer signature

Signature of explainer:

Adapted from standard MR Consent form used at Groote Schuur Hospital, Form PD 280



CONSENT FORM
Volunteer consent to undergo participation in experiment

The following information is very important to ensure your safety and to prevent any interference with the Magnetic Resonance (MR) Scan.

Please answer all the questions and mark each row with an X

Weight – kg 71

Date: 26/10/2009

Question	Yes	No	Unsure
Pacemaker		✓	
Aneurysm clip		✓	
Artificial valve		✓	
Vena cava filter		✓	
Prosthesis		✓	
Cochlear implant		✓	
Shrapnel in eye/ body		✓	
Neurostimulator		✓	
Any other implant (eg screws, plates, rods)		✓	
Are you pregnant?		✓	
Are you claustrophobic?		✓	
Other		✓	

I hereby acknowledge that the potential risks of the examination have been explained to me.

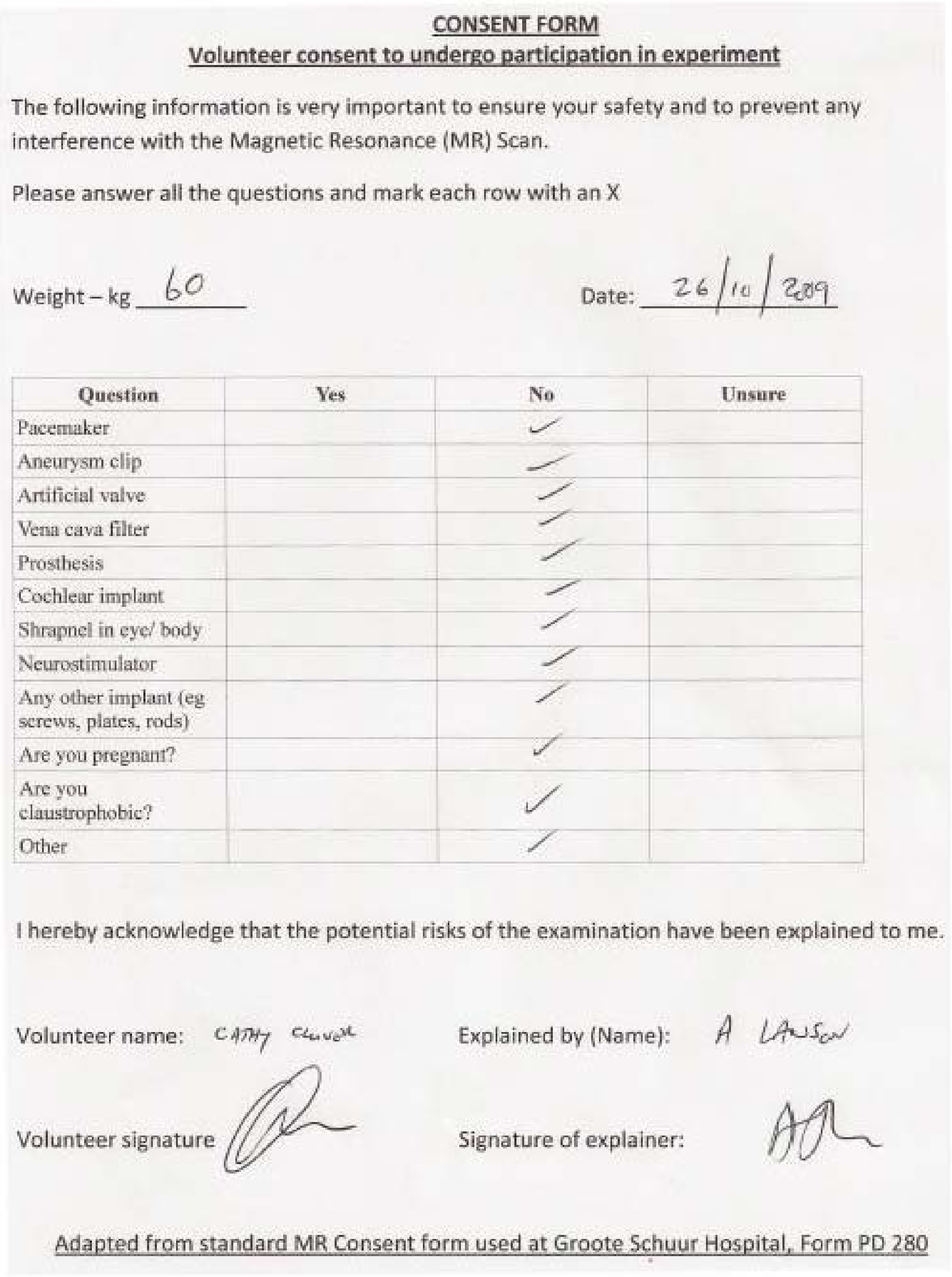
Volunteer name: UMAR EDWARDS

Explained by (Name): A. LAWSON

Volunteer signature

Signature of explainer:

Adapted from standard MR Consent form used at Groote Schuur Hospital, Form PD 280



CONSENT FORM

Volunteer consent to undergo participation in experiment

The following information is very important to ensure your safety and to prevent any interference with the Magnetic Resonance (MR) Scan.

Please answer all the questions and mark each row with an X

Weight – kg 60

Date: 26/10/2009

Question	Yes	No	Unsure
Pacemaker		✓	
Aneurysm clip		✓	
Artificial valve		✓	
Vena cava filter		✓	
Prosthesis		✓	
Cochlear implant		✓	
Shrapnel in eye/ body		✓	
Neurostimulator		✓	
Any other implant (eg screws, plates, rods)		✓	
Are you pregnant?		✓	
Are you claustrophobic?		✓	
Other		✓	

I hereby acknowledge that the potential risks of the examination have been explained to me.

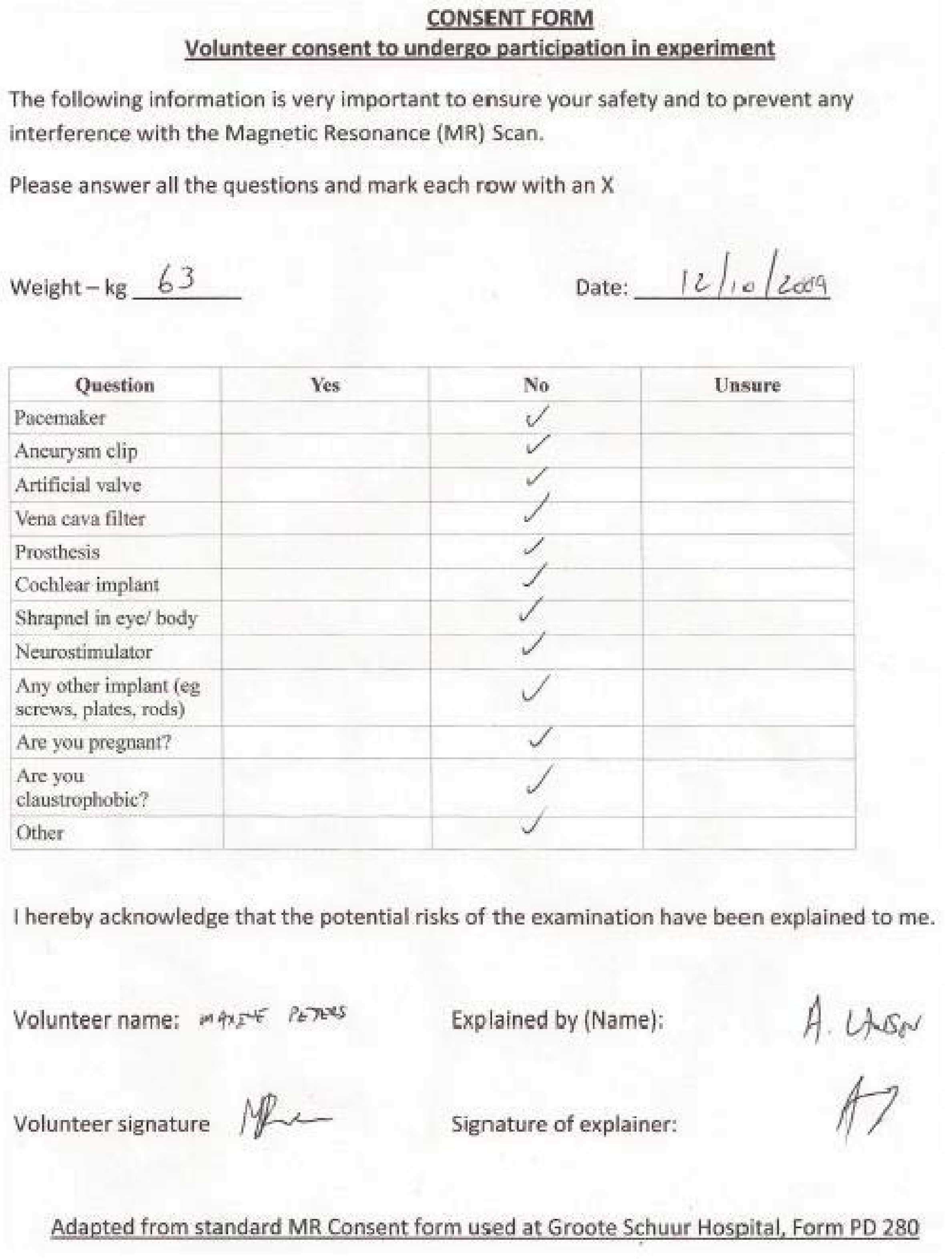
Volunteer name: CATHY CLUVER

Explained by (Name): A LAWSON

Volunteer signature

Signature of explainer:

Adapted from standard MR Consent form used at Groote Schuur Hospital, Form PD 280



CONSENT FORM

Volunteer consent to undergo participation in experiment

The following information is very important to ensure your safety and to prevent any interference with the Magnetic Resonance (MR) Scan.

Please answer all the questions and mark each row with an X

Weight – kg 63

Date: 12/10/2009

Question	Yes	No	Unsure
Pacemaker		✓	
Aneurysm clip		✓	
Artificial valve		✓	
Vena cava filter		✓	
Prosthesis		✓	
Cochlear implant		✓	
Shrapnel in eye/ body		✓	
Neurostimulator		✓	
Any other implant (eg screws, plates, rods)		✓	
Are you pregnant?		✓	
Are you claustrophobic?		✓	
Other		✓	

I hereby acknowledge that the potential risks of the examination have been explained to me.

Volunteer name: MAXENE PETERS

Explained by (Name): A. Larson

Volunteer signature

Signature of explainer:

Adapted from standard MR Consent form used at Groote Schuur Hospital, Form PD 280

Guide for authors Journal of Biomechanics

Affiliated with the American Society of Biomechanics, the European Society of Biomechanics, the International Society of Biomechanics, the Japanese Society for Clinical Biomechanics and Related Research and the Australian and New Zealand Society of Biomechanics.

The following types of manuscripts can be submitted for publication:

1. ***Surveys,*** normally 4000 to 6000 words (by invitation from the Editor only).
2. ***Original Articles,*** normally 2000 to 3000 words (3000 words approximately equals the content of 12 double-spaced manuscript pages with additional space for 8 to 10 figures or tables), although longer articles may occasionally be considered by the editors in special circumstances. Original articles typically explore some explicit biological hypothesis or report original but substantial observations or data of broad utility. Conceptually novel experimental or computational methods may be submitted as Original Articles when their relevance and importance for research of biological questions is demonstrated or otherwise emphasised in the text.
3. ***Perspective Articles,*** typically in the range of 500-2000 words. These manuscripts will explore controversial yet important themes, allowing expression of particular views or speculations, yet based on a solid understanding of published scientific information. Currently, such articles are by invitation only.
4. ***Short Communications,*** in the range of 500 to 1500 words, reporting preliminary observations, new interpretations of old data, simple new techniques or devices, or points of historical interest.
5. ***Book Reviews,*** normally no longer than 1000 words (by invitation from the Book Review Editor only).
6. ***Letters to the Editor*** normally no longer than 1000 words.

What information to include with the manuscript

1. Having read the criteria for submissions, authors should specify in their letter of transmittal, and on the title page, whether they are submitting their work as an Original Article, Perspective Article, Short Communication, or a Letter to the Editor.

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