Version 1.2

Non-invasive molecular imaging to determine the hemoglobin and collagen content in muscles before and after physical exercise and over time (MSOT_muscles)

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Sponsor:
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Study protocol

MSOT_muscles

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1. Table of contents

2. Study title, version number, version date

Study title

Non-invasive molecular imaging to determine the hemoglobin and collagen content in muscles before and after physical exercise and over time.

Version number

Version 1.2

Version date

23.04.2019

3. Summary of the project

Since 2017, a Multispectral Optoacoustic Tomograph (MSOT) with an extended emission spectrum is available to the University Hospital Erlangen through a DFG large-scale equipment funding. For MSOT imaging, similar to conventional sonography a transducer is placed on the skin, and instead of sound, energy is applied to the tissue by means of pulsed laser light. This leads to a constant change of minimal expansion and contraction of individual tissue components or molecules. The resulting sound waves can then be detected by the same device. Previous studies have already shown that the quantitative determination of haemoglobin can provide information on blood flow and inflammatory activity in chronic inflammatory bowel diseases. In the extended spectrum other markers such as collagens and lipids can be detected in addition to haemoglobin and its oxygenation status. We were able to show with our experimental preliminary work and a large animal pig model that an in vivo quantification of collagen is possible. In the first application of MSOT in children with Duchenne muscular dystrophy, the measured collagen content correlated with the clinical function tests. In summary, we could show that the molecular muscle structure quantified by MSOT allows the establishment of new, age-independent, non-invasive molecular biomarkers for disease assessment and therapy monitoring in patients with DMD.

Neuromuscular diseases often present as early as in neonatal age and manifest themselves in muscular hypotonia and weakness. Associated diseases are caused by numerous pathologies in the central nervous system (brain and spinal cord), the peripheral nervous system or skeletal muscle. X-linked progressive muscular dystrophy of the Duchenne type (DMD) is one of the most common progressive muscular diseases of childhood with an incidence of 1:3500 male newborns and is primarily associated with a reduced life expectancy. Around the age of 4-5 years, motor problems manifest themselves in everyday life with typical signs of proximal muscle weakness and laboratory chemical increase of the muscle enzyme (creatinine kinase, CK). Within a few years, relevant muscle and tendon shortenings develop, leading to joint malpositions, instabilities, and consecutively to scoliosis and loss of walking around the age of 10. Supportive therapy measures cannot curatively influence complications and progression of the disease. Pathogenetically, there is a deficiency of dystrophin, a structural protein of the sarcolemma, which is caused by mutations (mostly deletions) of the dystrophin gene (Xp21.3-p21.2). The consequence of dystrophin deficiency is necrosis of the muscle cells which are replaced by connective tissue and fat. The 6-minute walk test (6-MWT) is usually used to quantitatively assess the disease progression as well as the effects of therapy. In this test, the patient need to walk as much distance as possible within 6 minutes. However, this test is dependent on cooperation and is usually not successful in the very young patients. The effects of new gene therapeutic therapies (e.g. PTC 124) are currently still being

assessed with the help of this clinical function test. Sensitive, non-invasive methods for measuring muscle degeneration at an early stage and muscle function during the course of the disease are therefore of great clinical and scientific importance.

The MSOT data collected so far seem promising to enable therapy monitoring in DMD patients. Preliminary work by other groups has already demonstrated a very good precision and reproducibility of MSOT. However, it has not yet been investigated to what extent 1) previous physical activity, 2) different measurement positions of the muscle, 3) different examination dates and 4) intra- and interrater variability influence the measurement results. Especially molecular changes immediately after the 6-MWT are relevant for studies in muscle patients with DMD.

In this first pilot study, the aim is to investigate in healthy adults whether increased exercise alters haemoglobin values, the collagen content remains constant and repeated measurements at different muscle positions by different investigators provide consistent measurement results over time. This information is essential for further studies of children with neuromuscular disorders to properly evaluate a possible response to therapy.

4. Responsabilities

Study lead

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Sponsoring

Children's Hospital Erlangen, Else-Kröner-Fresenius Foundation (Else-Kröner-Fresenius Memorial Scholarship Ferdinand Knieling)

5. Scientific background

Since 2017, the University Hospital Erlangen (Medical Clinic 1, Pediatric and Adolescent Clinic) has a Multispectral Optoacoustic Tomograph (MSOT) funded by the DFG. This allows non-invasive, quantitative imaging of the composition of target tissues in the non-sedated child, comparable to sonography. This is of particular importance in this patient group.

In MSOT, similar to conventional sonography, a transducer is placed on the skin and instead of sound, energy is supplied to the tissue by means of pulsed laser light. This leads to a constant change of minimal expansions and contractions (thermoelastic expansion) of individual tissue components or molecules. The resulting sound waves can then be detected by the same examination unit. Previous studies have shown that the quantitative determination of hemoglobin can be used to obtain information on blood flow and inflammatory activity in the intestine from patients with Crohn's disease (Waldner, Knieling et al. 2016, Knieling, Neufert et al. 2017) (Waldner 2016, Knieling 2017). In the newly configured device (Acuity Echo, iThera Medical GmbH, Munich, prototype) an extended spectrum of laser light can be used, which ultimately not only enables the detection of haemoglobin and its oxygenation stages, but also the detection of further markers such as collagen and lipid. This principle has already been successfully used in a follow-up study on pediatric patients with Duchenne muscular dystrophy (Regensburger, Fonteyne et al., submitted for publication, ESMI Young Investigator Award 2019).

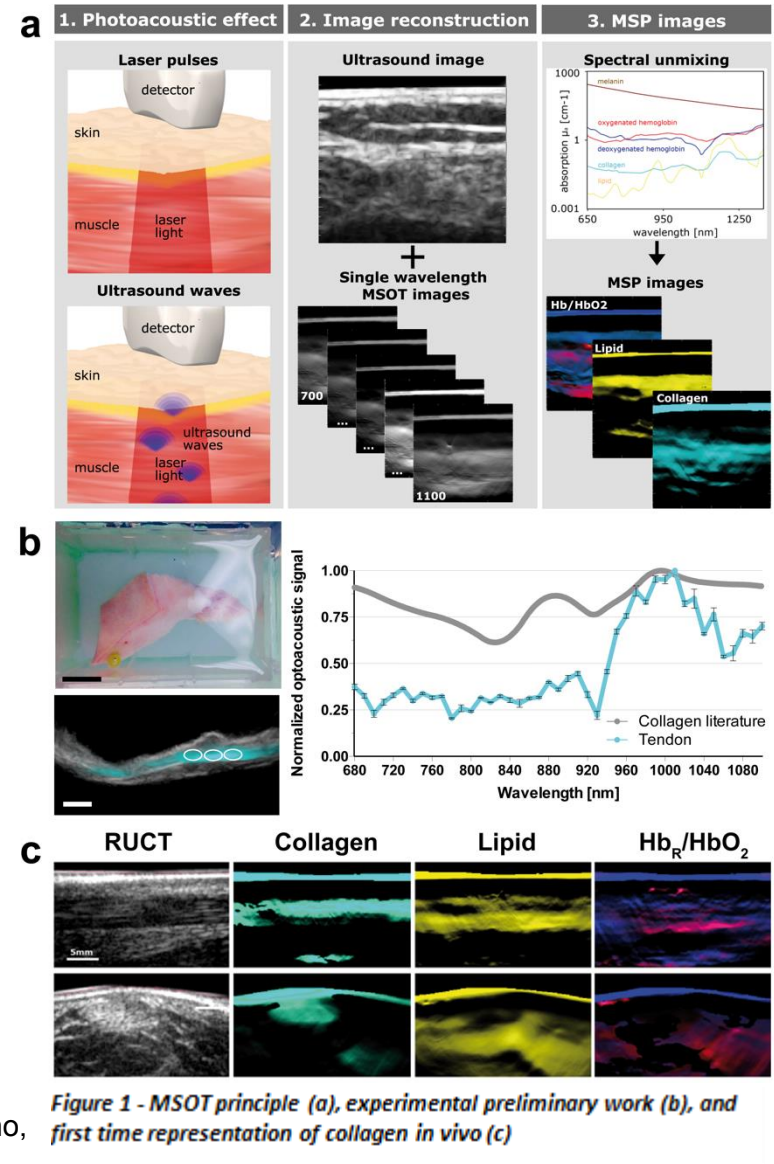

Figure 1 - MSOT principle (a), experimental preliminary work (b), and first time representation of collagen in vivo (c)

We were able to show by our experimental preliminary work and a large animal pig model that an in vivo quantification of collagen is possible. In the first application of MSOT in children with Duchenne muscular dystrophy, the measured collagen content correlated with the clinical function tests. In summary, we could show that the molecular muscle structure quantified by MSOT allows the establishment of new, age-independent, non-invasive molecular biomarkers for disease assessment and therapy monitoring in patients with DMD.

Neuromuscular diseases often present as early as in neonatal age and manifest themselves in muscular hypotonia and weakness. Associated diseases are caused by numerous pathologies in the central nervous system (brain and spinal cord), the peripheral nervous system or skeletal muscle. X-linked progressive muscular dystrophy of Duchenne type (DMD) is one of the most common progressive muscular diseases of childhood with an incidence of 1:3500 male newborns and is primarily associated with a reduced life expectancy (Mendell and Lloyd-Puryear 2013). From the age of 4-5 years, motor problems manifest in everyday life with typical signs of proximal muscle weakness and laboratory chemical increase of the muscle enzyme (creatinine kinase, CK). Within a few years, relevant muscle and tendon shortenings develop, leading to joint malpositions, instabilities, and scoliosis and loss of walking around the age of 10. Supportive therapy measures cannot curatively influence complications and progress of the disease. Pathogenetically, there is a deficiency of dystrophin, a structural protein of the sarcolemma, which is caused by mutations (mostly deletions) of the dystrophin gene (Xp21.3-p21.2). The consequence of dystrophin deficiency is necrosis of the muscle cells which are replaced by connective tissue and fat (Hoffman, Brown et al. 1987, Klingler, Jurkat-Rott et al. 2012, Mercuri and Muntoni 2013). The 6-minute walk test (6-MWT) (McDonald, Henricson et al. 2010, McDonald, Henricson et al. 2010, Bushby, Finkel et al. 2014, Mendell, Goemans et al. 2016, McDonald, Campbell et al. 2017) is usually used for the quantitative assessment of the disease progression as well as therapy effects. Here the patient needs to walk the longest distance possible within 6 minutes.

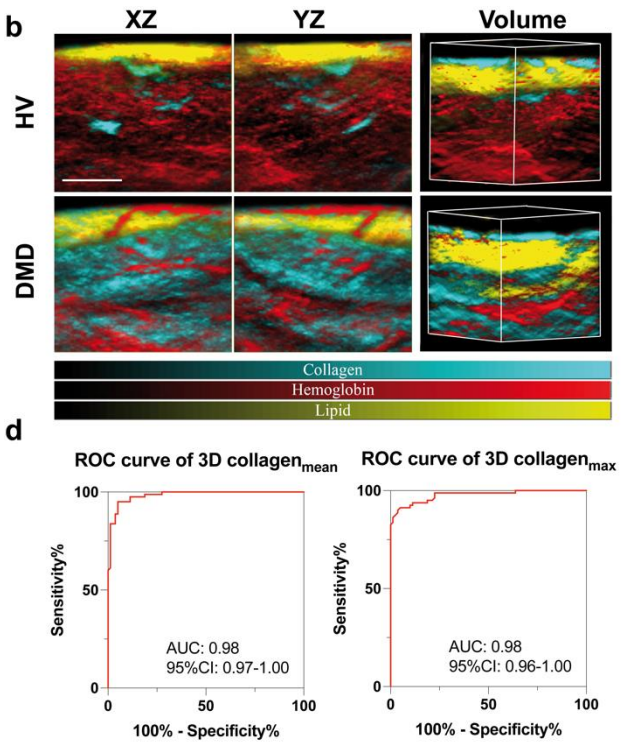

Figure 1 – Feasibility of 3D MSOT image.

However, this test is dependent on cooperation and is usually not successful in very young patients. In addition, daily fluctuations in the patients' function and strength as well as learning effects can influence the results. A problematic and limiting factor for the use of the 6MWT as a reliable marker is that progressive muscle degeneration is only detected functionally at a late stage. MRI studies to characterize the degenerative changes in early-stage skeletal muscles show promising results in order to find quantitative measures to determine edematous changes and fatty degeneration (Arpan, Willcocks et al. 2014, Bonati, Hafner et al. 2015, Glemser, Jaeger et al. 2017, Barnard, Willcocks et al. 2018). For clinical use, the required sedation of the patients may be limiting for the feasibility of MRI diagnostics. The highly sensitive assessment of therapeutic effects is expected to become increasingly important in the future. In the meantime, the detection of a nonsense point mutation leading to a premature stop codon and thus to the premature termination of protein synthesis has therapeutic consequences. For walkable boys with DMD with a proven nonsense mutation, a gene therapy approach with PTC 124 (Translarna®, Ataluren, PTC Therapeutics) has been available since 2016 which leads via ribosomal read-through to the formation of functionally active full-length dystrophin and to a significant improvement of muscle function (Bushby, Finkel et al. 2014, McDonald, Campbell et al. 2017). Methods for assessing the efficacy are controversially discussed and are not standardized established in clinical practice. Sensitive, non-invasive methods for recording muscle degeneration at an early stage and muscle function during its course are therefore of great clinical and scientific importance.

The MSOT data collected so far seem promising to enable therapy monitoring in DMD patients. Preliminary work by other groups has already demonstrated a very good precision (Joseph, Tomaszewski et al. 2017) and reproducibility (Helfen, Masthoff et al. 2019) of MSOT measurements (with a technically slightly different prototype). However, no investigations have been carried out so far:

- to what extent physical activity influences the measurement results
- the extent to which the MSOT values vary within a muscle (different muscle positions)
- whether the measured values change over time, and
- whether the intra- and interrater differences also apply to this MSOT prototype

In this first pilot study, the aim is to investigate in healthy adults whether increased exercise changes the haemoglobin values, the collagen content remains constant and repeated measurements at different positions and by different examiners over time provide consistent measurement results. This information is essential for planning further studies in children with neuromuscular disorders, and to be able to evaluate a possible response to therapy.

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6. Study objectives

The aim of the proposed study is a longitudinal comparison of muscle tissue composition based on Multispectral Optoacoustic Tomography (MSOT) of healthy, adult subjects, before and after physical exercise.

Primary/secondary objectives and/or hypotheses

Hypotheses:

- The quantitative amount of hemo/myoglobin signal in muscles of healthy volunteers determined by MSOT differs *before and after the 6-minute walk test*.
- The quantitative amount of oxygenated/deoxygenated hemo/myoglobin signal in muscles of healthy volunteers determined by the MSOT differs *before and after the 6-minute walk test.*
- The quantitative amount of collagen signal in muscles of healthy volunteers determined by MSOT does not differ *before and after the 6-minute walk test.*
- The quantitative amount of hemo/myoglobin signal in muscles of healthy volunteers determined by the MSOT does not differ when *the follow-up is measured after 2 weeks.*
- The quantitative proportion of oxygenated/deoxygenated hemo/myoglobin signal in muscles of healthy volunteers determined by MSOT does not differ when *the follow-up is measured after 2 weeks.*
- The quantitative proportion of collagen signal in muscles of healthy volunteers determined by MSOT does not differ when *the follow-up is measured after 2 weeks.*
- The quantitative proportion of hemo/myoglobin signal in muscles of healthy volunteers determined by MSOT does not differ *within a muscle* of healthy volunteers.
- The quantitative proportion of oxygenated/deoxygenated hemo/myoglobin signal determined by MSOT does not differ *within one muscle* of healthy volunteers.
- The quantitative fraction of collagen signal determined by MSOT does not differ *within one muscle* of healthy subjects.
- The quantitative amount of hemo/myoglobin determined by MSOT does not differ *between two measurements by the same examiner.*
- The quantitative amount of oxygenated/deoxygenated hemo/myoglobin determined by the MSOT does not differ *between two measurements by the same examiner*.
- The quantitative amount of collagen determined by the MSOT does not differ *between two measurements by the same examiner*.
- The quantitative amount of hemo-/myoglobin determined by the MSOT does not differ *between measurements made by two different examiners*.

- The quantitative amount of oxygenated/deoxygenated hemo-/myoglobin determined by the MSOT does not differ *between measurements performed by two different examiners.*
- The quantitative amount of collagen determined by the MSOT does not differ *between measurements made by two different examiners.*

Primary objective:

- Comparison of the quantitative proportion of hemo/myoglobin and collagen signal in muscle tissue of healthy subjects before and after physical exercise (6-MWT) determined by MSOT, as well as over time.

Secondary objectives:

- Comparison of the quantitative amount of hemo/myoglobin and collagen signal determined by MSOT in muscle tissue of healthy volunteers at timepoint 1 and timepoint 2 after two weeks
- Comparison of the quantitative proportion of oxygenated/deoxygenated hemo/myoglobin signal in muscle tissue of healthy volunteers at timepoint 1 and timepoint 2 after two weeks
- Comparison of the MSOT-determined quantitative amount of hemo/myoglobin and collagen signal at three muscle positions of two muscles (quadriceps femoris and gastrocnemius)
- Comparison of the quantitative proportion of oxygenated/deoxygenated hemo/myoglobin signal determined by MSOT in three muscle positions of two muscles (quadriceps femoris and gastrocnemius)
- Comparison of the quantitative proportion of hemo/myoglobin and collagen signal determined by MSOT in muscle tissue of healthy female and male volunteers
- Comparison of the quantitative proportion of oxygenated and deoxygenated hemo/myoglobin signals determined by MSOT in muscle tissue of healthy female and male volunteers
- Correlation of the hemo/myoglobin and collagen signal determined with MSOT with the 6-MWT
- Correlation of the oxygenated/deoxygenated hemo/myoglobin signal determined by MSOT with the 6-MWT
- Comparison of the quantitative amount of hemo/myoglobin and collagen signal determined by MSOT between two independent examiners

- Comparison of the quantitative amount of hemo/myoglobin and collagen signal determined by MSOT between two images of one examiner

Study type

Since no data exists to date to support the hypothesis of this study, this is an exploratory study.

7. Target parameters

All measurements with MSOT are performed at three muscle positions of the proximal and distal lower extremity muscles in lateral comparison (right - left, leg proximal: Musculus quadriceps, distal: Musculus gastrocnemius) by two different examiners, in healthy subjects, at two different timepoints.

Primary target:

Quantitative hemo/myoglobin and collagen signal (in arbitrary units)

This target is measured non-invasively by MSOT.

Secondary targets:

Quantitative lipid signal (in arbitrary units)

Muscle oxygenation (in arbitrary units)

This target is measured non-invasively by MSOT.

6-minute walk test (6-MWT)

This target value is clinically determined at presentation.

Age

Gender

Weight

Skin colour

Ethnic background

Underlying diseases

Current medication

This information is collected for the study at presentation.

8. Study design

Monocentric / multicentric

This is a monocentric study with prospective data acquisition (Investigator Initiated Trial, IIT).

Study arms: intervention/control

Interventions are not planned. A comparison is made between healthy volunteers. The study procedure is identical for all test subjects.

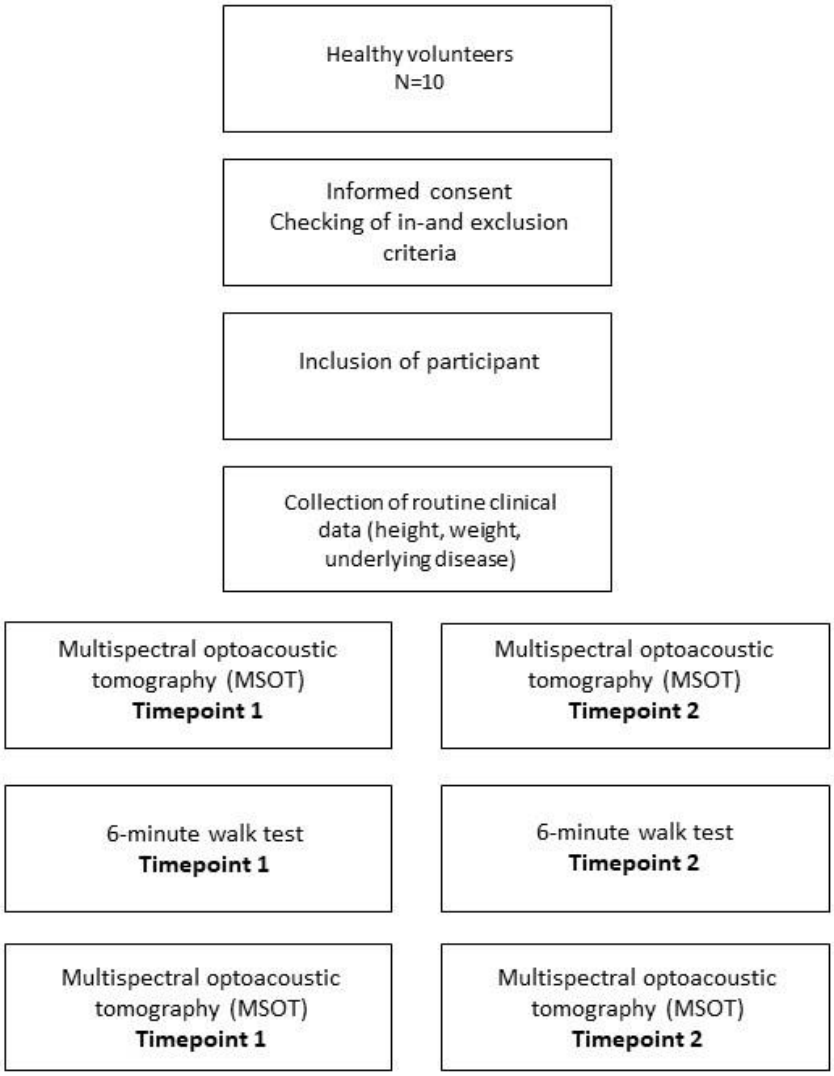
Randomization

Randomization is not planned. These is no allocation to groups.

Blinding

Blinding for the study is not possible due to the study design. Blinding is performed during the measurement and analysis of the data. Blinding of the test subjects is not necessary.

Graphical presentation of study design



9. Study population

Inclusion and exclusion criteria

Inclusion criteria:

Healthy volunteers:

- Adult (>18 years of age) participants

Exclusion criteria:

Participants:

- anamnestic or other signs of myopathy
- pregnancy
- Tattoo on skin to be examined
- missing consent form

Participant number

As this is a pilot study, it is not possible to calculate the exact number of cases. It is planned to examine a total of 10 healthy volunteers.

Recruitment routes and measures

Healthy volunteers will be informed about the possibility to participate in the study. If they are willing to participate, they will be fully informed about objectives and methods (especially about the scientific/explorative character of the study), benefits and risks and the revocability of their participation in the study.

10. Study course

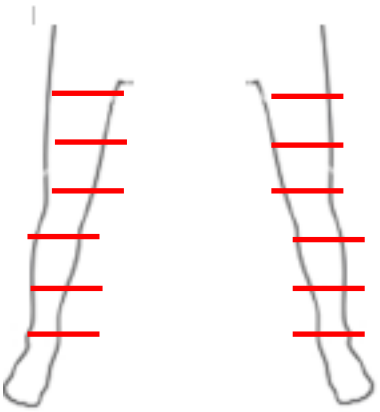
Procedure for informing about and obtaining consent

Participants can only be included in the study after a written declaration of consent has been given. The written declaration of consent requires that the volunteers are informed orally and in written form about the objectives and methods (including the scientific-explorative character of the study), benefits and risks as well as the revocability of their participation in the study. The information is provided by means of comprehensible proband information sheets. By giving their written consent, the volunteers declare that they agree to the collection and storage of data relevant to the study and their verification by monitoring or authorities. It must be clearly communicated to the study participant that a withdrawal of consent is possible at any time and without any disadvantage. Furthermore, all study participants/test persons are informed that this study is a purely scientific study without any current diagnostic or therapeutic benefit.

The original of the consent form is kept in the study folder at the study site. The volunteer receives a copy of the patient information and consent form. The proband information and the consent form can be found in the appendix of this study protocol.

Measurements

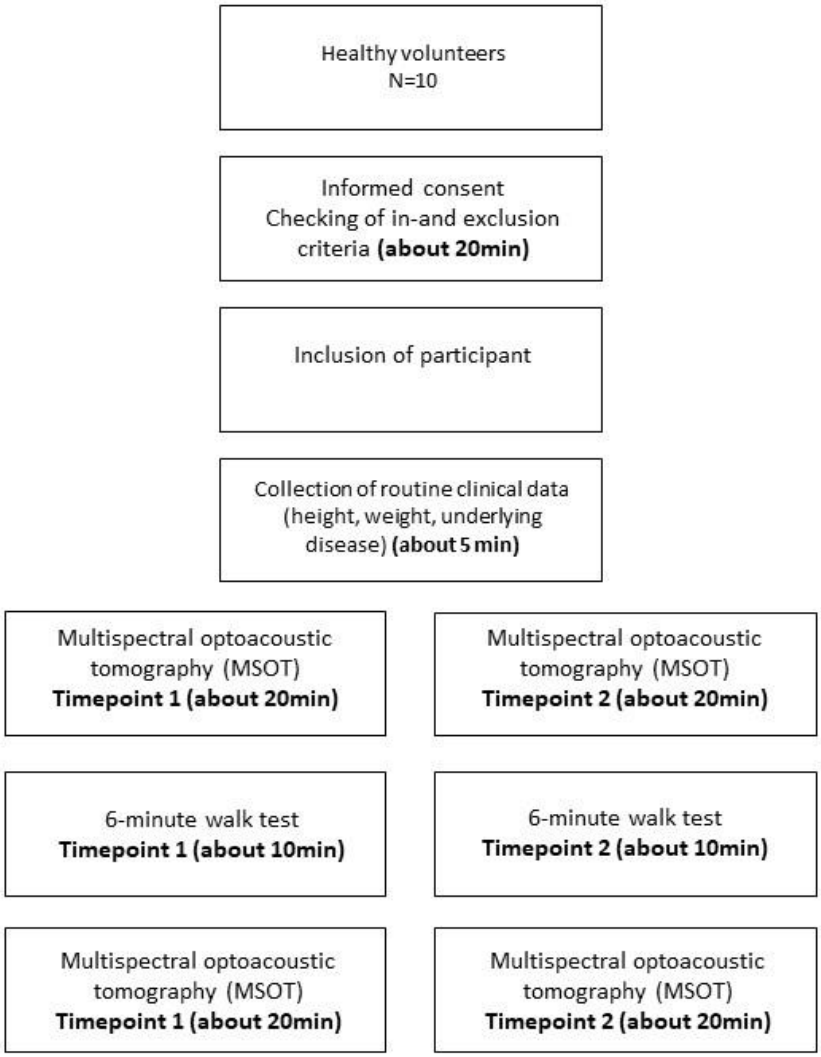
After informing of the subjects, all study participants are imaged by MSOT at 3 positions of 2 anatomical muscle regions on both sides (right and left): upper/lower leg, on predefined muscle groups (leg proximal: Musculus quadriceps, distal: Musculus gastrocnemius). The examination is performed by two independent examiners directly one after the other. The examination is performed analogous to sonography over the corresponding skin layers without further invasive procedures. The anatomical region can be localized by means of built-in B-scan sonography; the corresponding optoacoustic signals can be recorded in parallel. Each examiner performs two repetitive scans per muscle position. The duration per anatomical region is limited to 5 minutes; this corresponds to a maximum of 20 minutes for both lower extremities. The subjects can remain in a relaxed body position during the examination; assistance in the form of breathing manoeuvres or similar is not necessary. Subsequently, a 6-minute walk test is performed to test muscle activation. Afterwards another examination with MSOT is carried out analogous to the above mentioned procedure. The same procedure is repeated after 14 days.



Recording of target parameters

- Non-invasive in-vivo measurement of hemo/myoglobin, collagen and lipid content/signal and oxygenation by MSOT
- Assessment of the 6-minute walk test
- Determination of routine data (height, weight, underlying diseases, medication)

Time schedule and study duration for the individual volunteer:



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The duration of the study participation is about 125 minutes for the individual volunteer. At timepoint 1 the duration of study participation is 75 minutes. Approximately 20 minutes are allotted for the education of study participants, approximately 5 minutes for the collection of routine clinical data, 10 minutes for clinical (routine) testing using 6-MWT, and 40 minutes for the actual examination. At timepoint 2, participation time for the study is about 50 minutes. The clinical (routine) test using 6-MWT takes 10 minutes, and 40 minutes for the actual examination.

Total study duration

Depending on the number of patients, the expected total duration of the study until the inclusion of the last patient is approximately 6 months.

11. Risk-benefit analysis

All study related risks

Based on the classification criteria for medical devices (Directive 93/42/EEC, Annex IX), the optoacoustic system of iThera Medical corresponds to Class IIa:

- Active diagnostic device
- non-invasive
- Temporary use (<60 min)

No CE certification is available for this research device (current type designation according to imprint: Acuity Echo). A conformity assessment procedure in the sense of the MPG is not intended or planned by the manufacturer at the present time. It is therefore a purely scientific pilot study. There is no dependency on the manufacturer, all diagnostic and analytical procedures are available to the study directors on site.

Adherence to energy levels

The laser safety and maximum permitted radiation dose for irradiation with laser pulses is regulated in the laser standards ANSI and IEC 60825. The MSOT system meets these standards and therefore remains below the MPE (maximum permissible exposure) limits for skin irradiation and is therefore considered safe.

Temperature increases due to MSOT in tissue

Optoacoustic imaging does not result in any significant temperature increase in the tissue. The absorption of a laser pulse in the tissue results in a local transient temperature increase of a few millikelvin. Depending on the duration of the examination and the skin type of the patient, temperature increases occur typically in the range of less than one degree Kelvin.

Histological changes in tissue

Histological changes in the target tissue and surrounding structures are neither expected nor have they been observed in previous preclinical and clinical studies.

Slight, reversible redness or warming might occur in very sensitive skin.

Such side effects are to be noticed at any time by the test person or doctor; the examination can then be interrupted or aborted. In any case, no irreversible damage is to be expected.

In general, the near infrared light used in the MSOT can lead to retinal damage if the eye is irradiated. In order to prevent this, test participants and examiners will wear appropriate laser safety glasses during the examination.

Since the data obtained is not used to interpret diagnostic results, there is no risk of possible misdiagnosis or incorrect display of data in this exploratory pilot study.

No other risks exist for this study, nor have we described any risks based on our own preliminary data.

Benefits associated with the study

The data obtained in this study can provide important information about the changes of quantitative MSOT signal in muscles due to physical exercise, as well as over time. The quantifiable differences could be important for future planning of further studies and the avoidance of errors in the study design. Additionally, first longitudinal data are essential to design appropriate studies.

Termination criteria

Termination criteria for the individual participant:

Participation in the study is terminated if there is noticeable warming or reddening of the skin. The examination time per anatomical region is limited to 5 minutes, making these events highly unlikely.

Due to the short duration of the study participation, no other discontinuation criteria are planned.

Termination criteria for the whole study:

A termination of the entire study is not planned.

Statement on medical justifiability

Given to previous experience, especially with children, the risk of undesirable events is considered as extremely low.

No serious incident has been reported so far, neither at our site - nor in the literature. The majority of reported (foreseeable) problems are related either to the use of ultrasound gel for examination or the need to wear eye protection. The use of filter glass also explains the reported red visibility. This phenomenon was reversible within seconds. Table 1 shows the reported events from our study (MSOT_DMD, 67_18 B).

	Muscular dystrophy Duchenne N=10	Healthy volunteer N=10
Reversible adverse events- no.(%)		
Pressure of safety goggles	2 (20%)	
Coolness of Ultrasound-gel		4 (40%)
Red cast view		1 (10%)
Serious adverse events- no.(%)	0 (0%)	0 (0%)

Table 1 – Adverse events

Only measurements of extremities and no central organs are examined in this study - this leads to a further significant reduction of a possible residual risk. Particularly in light of the background of completely new therapeutic approaches, we hope that this method will provide us with a child-friendly diagnostic tool for the treatment of complex muscular diseases.

12. Biometrics

Explorative study: explanation of the statistical methodology, justification of the selected number of cases

Case number calculation:

As this is a pilot study and no information on the expected differences between the different groups is available yet, no case number calculation was performed. The number of cases given represents an estimate.

Statistical methods:

The data are given as mean value with standard deviation. Correlations are specified with the parametric Pearson correlation coefficient (R). According to the distribution between timepoint 1 and timepoint 2, the differences of the mean values are statically examined with a parametric T-test. In all analyses, an error level of <0.05 is considered statically significant.

13. Data management und and data protection

Data acquisition and storage

All raw data, such as patient files, are source documents. Their availability is ensured for routine monitoring. The participation of the individual patients or test persons in the study is documented. The study leader maintains an independent list for the identification of the participating patients. This list containes the names and date of birth as well as the date of examination and pseudonymisation codes of the patients and subjects. The study leader is responsible for the quality of data collection and storage. The data storage (complete data) takes place on computers or specially designed network drives of the University Hospital Erlangen. The raw imaging data (no patient-related data) are stored on dedicated servers of iThera Medical GmbH.

Pseudonymisation

Prior to a scientific analysis of the materials and data of this study, all information will be pseudonymized according to the guidelines of the Federal Data Protection Act.

Data transfer

A transfer of the data or biological material is not foreseen in this study and will not take place. The study results can be published anonymously, but it will not be possible to draw conclusions about the identity of the participating persons. The data is stored for 10 years and then destroyed.

Revocation, data deletion

If the declaration of consent is revoked, data collected up to this point can be taken into account. The patient has the right to demand that the data be destroyed, provided that legal provisions do not prevent such destruction.

14. Handling of biomaterials

No biomaterials are obtained.

15. Insurance

The participants of the study are insured via the group contract of the CCS Erlangen.

16. Signatures

Dr. med. Ferdinand Knieling
Study director

Dr. med. Adrian Regensburger
Study directors

Prof. Dr. Regina Trollmann
Study director

Prof. Dr. h.c. Dr. med. W. Rascher
Clinic director

Prof. Dr. med. Maximilian Waldner
Technical implementation