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TITLE: Targeted nutritional approach to improve muscle function and physical activity by restoring metabolic deregulations during recovery from sepsis

PRINCIPAL INVESTIGATOR: Nicolaas Deutz

CONTRACTING ORGANIZATION: Texas A&M University, College Station, TX

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6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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14. ABSTRACT

Purpose:The major goal is to test the hypothesis that a unique formulation based on essential amino acids (EAA) will improve physical activity, and involuntary isometric skeletal muscle strength faster during the recovery of a sepsis event.
Scope:The proposed study in a catheterized pig model recovering from sepsis is innovative because, a) the targeted nutritional supplementation is a novel approach to attenuate tissue breakdown in sepsis and improves functional outcome and restores muscle mass, and it provides insights into sepsis-induced severe tissue breakdown and physical outcome. The use of innovative, stable tracer methodology to measure metabolic fluxes within and across muscle, enables quantification of all metabolic endpoints. The results of the study will have a positive impact by providing the basis to develop novel cost-effective nutritional approaches for patients recovering from sepsis to improve recovery and rehabilitation. It has a strong justification because of its rapid translation into clinical application.
Year 2 Achievement: We performed studies in 32 animals with some delay due to Covid-19, change of animal vendor, and animal supply. We implemented/validated the analysis of the animal activity and implemented the analysis of the muscle strength responses. No changes needed to current approach.

15. SUBJECT TERMS
Sepsis, nutritional support, essential amino acids, muscle strength, pig, protein synthesis, protein breakdown, muscle breakdown.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
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INTRODUCTION

As the endotoxin-induced sepsis-like state is not comparable to real life human sepsis, we have developed a translational model in which sepsis is induced by continuous intravenous infusion with live bacteria (Pseudomonas aeruginosa) using a pig model. In this sepsis model, we observed complex multiorgan protein metabolic disturbances including muscle protein catabolism{Ten Have, 2015, S33}{Ten Have, 2017, 1-301}{Ten Have, 2019, G755-G762}, ultimately leading to bacterial translocation, increased cytokine release, and stimulated muscle breakdown. Our approach is to study the effects of targeted dietary EAA enriched nutritional supplementation versus a sham-control on muscle function and physical outcome in the recovery phase from sepsis (primary endpoint). Muscle function and physical outcome will be related to net protein synthesis on whole body and muscle level (secondary endpoint). In a randomized, sham controlled, blind (for nutritional intervention) 2 group design, we will use involuntary isometric skeletal muscle strength and physical activity measurements for functional outcome. Innovative, stable tracer technologies will be used to quantify metabolic endpoints like muscle net protein synthesis and muscle mass.

KEYWORDS

Sepsis, nutritional support, essential amino acids, muscle strength, pig, protein synthesis, protein breakdown, muscle breakdown.

ACCOMPLISHMENTS

What were the major goals of the project?

The major goal of the project is to test the hypothesis that a unique formulation based on EAA will improve physical activity, and involuntary isometric skeletal muscle strength faster during the recovery of a sepsis event.

What was accomplished under these goals in Year 2?

Major Task 1: Pig studies*:*

Subtask 1: Submit documents for IACUC approval: Milestone Achieved in year 1, 100% completed. No changes needed to be made concerning the approach.

Subtask 2: Implementation of logistics for monitoring physical activity and muscle strength measurement (buying testing equipment) and other surgery/animal care/pharmaceutical compounding/ analytical related preparations. Milestone achieved in year 1, 100% completed: Logistics are in place.

Subtask 3: Performing animal studies. 62 animals, 4 animals/6 weeks. Planned between month 7-30. Planned milestone % completion in at the end of year 2: 75%. However 32 of the 62 planned animals are completed (= 53% completed)**.** Delay was due to Covid-19 university restrictions, change of animal vendor and animal supply.

Major Task 2: Analysing specimen - Activity (Video) monitoring

Subtask 1: For metabolic analysis. Analysis specimen with Mass Spectrometry. Planned between month 12-30. Planned milestone % completion at the end of year 2: 66%. Current % of completion: 15%: Getting mass spectrometry applications validated and in place. It resulted in the submission of an abstract of previous research in an animal model related to the used animal model in the present DOD-project (Title: Enhanced splanchnic extraction of nutritional arginine and lower citrulline production play a role in the reduced systemic availability of arginine in the early recovery phase of sepsis in the pig. See appendices for abstract 1). It is submitted to ESPEN 2022 conference (European Society for Clinical Nutrition and Metabolism). Batchwise specimen analysis will start in year 3. Delay was due to Covid-19 university restrictions and delays in animal studies, Major task 1, Subtask 3.

Subtask 2: Analysis of the video of the animal activity. Planned between month 12-30. Planned milestone % completion at the end of year 2: 66%. Current milestone % of completion 15%: We implemented/validated the analysis of the video's of the animal activity. Assessing physical activity an automatic video tracking system (Ethovision, Noldus) was tested and implemented together with foreighn batchles exchange student.

Logistics were tested. A significant amount of time was given to optimize/develop a new video tracking protocol for the pig in the home cage, resulting in a thesis of van Sas (not public available). A ethogram was developed and tested. Further finetuning is still needed. Delay was due to Covid-19 university restrictions and delays in animal studies, Major task 1, Subtask 3.

Subtask 3: Analysis of the muscle strength responses. Planned between month 12-30. Planned milestone % completion at the end of year 2: 66%. Current milestone % of completion 25%: Implemented the analysis of the muscle strength responses, resulting in the submission of an abstract (see abstract 2 in the appendices) and presentation (poster, see attach file to this report) at the 2022 Swine in Medical Research Conference: " Muscle Fatigue Measurements by Percutaneous Neural Stimulation in the Pig: Exploring a New Fatigue Protocol and Data Analysis.". Delay was due to Covid-19 university restrictions and delays in animal studies, Major task 1, Subtask 3.

Major Task 3: Data modeling - statistical data analysis
Subtask 1: Data modeling - statistical data analysis: Planned between month 31-36. Milestone completion 0% nothing to report. Will start in the third year.

Subtask 2: Writing scientific manuscripts: manuscript preparations ongoing. Milestone completion for 2-4 peer reviewed papers related to the goal of the present DOD-project: Current 20% completed. Manuscript preparations in the form of submitting abstracts and presenting/discussing research in (inter)national conferences started: An abstract were submitted to 2022 MHSRS - Military Health System Research Symposium with the title: "Targeted nutritional approach to improve muscle function and physical activity by restoring metabolic deregulations during recovery from sepsis: A study protocol of a pre-clinical trial." However not accepted due to overwhelming amounts of abstracts submitted to the symposium. See abstract 3 in appendices.

Also we presented 2022 Swine in Medical Research Conference. We submitted 3 abstracts with the titles: " Muscle Fatigue Measurements by Percutaneous Neural Stimulation in the Pig: Exploring a New Fatigue Protocol and Data Analysis." (see abstract 2 in the appendices and attached poster to this report), "Progressive decrease of the BIA Phase Angle in a *Pseudomonas Aeruginosa* induced sepsis swine model. A marker of severity?"(see abstract 4 in the appendices and attached poster to this report), "Evaluation of Continuous Glucose Monitors in Septic Swine." (see abstract 5 in the appendices and attached poster to this report). These were accepted for three poster presentations and presented June 12-14 in Madison, WI.

We submitted abstracts for the 2022 ESPEN conference (European Society for Clinical Nutrition and Metabolism) related to the present to the DOD project: with the titles: " Enhanced splanchnic extraction of nutritional arginine and lower citrulline production play a role in the reduced systemic availability of arginine in the early recovery phase of sepsis in the pig" (See appendices for abstract 1), and "Mainly muscle, jejunum, and lung fractional protein synthesis rates are related to whole-body protein synthesis in the pig" (See appendices for abstract 6).

What opportunities for training and professional development has the project provided?
In the second year, additional staff (Mackey) and 9 months full-time international exchange student (van Sas) were trained in the practical care and documentation needed to house the animals and ensure their well-being throughout the course of the study. We (Deutz, ten Have, Rice) have also on-boarded and trained new staff (Mackey) in the preparation, implementation, and analysis of samples/data generated during the metabolic study days. Mackey has also developed professional skills from formal surgical prep, maintenance of chronic catheters, and animal care during critical illness and recovery. Mackey and van Sas have developed technical skills in experimental design, behavior analysis, continuous glucose monitoring, metabolic tracking, use of isotopes in experimental design and data analysis. Rice and Mackey have developed skills to organize large

data bases and statistical analysis. The student research asistants (Beach, Blair, Cha, Smith) have developed skills to assist in preparation and performing complex animal studies

Assessing muscle function is one of the primary end points in this study. Dr. Peter Nghiem and his team (Alexis Rutledge) are considered one of the leading authorities on muscle testing in canine models for DMD and have published extensively in this field. Due to differences in muscle strength, a fatigue protocol (instead of an eccentric contraction decrement protocol, as performed in Dr. Nghiem's canine studies) was researched, instituted, and established for this current study. We (Deutz, ten Have) researched an improved data analysis protocol to ensure multiple muscle strength parameters can be extracted from the muscle testing test. Assessing physical activity an automatic video tracking system (Ethovision, Noldus) was tested and implemented by (ten Have, van Sas). Logistics were tested. A significant amount of time was given to optimize/develop a new video tracking protocol for the pig in the home cage, resulting in a thesis of van Sas. A ethogram was developed and tested. Further finetuning is still needed.

The entire team meets weekly to assess progress and discuss the logistics of the implementation of the protocol and address any issues that may arise during the test days.

Ten Have, Rice and Mackey had the opportunity to present/discuss results of the present DOD project with experts in the field during the 2022 Swine in Medical Research Conference (see appendices abstract 2,4 and 5, and attached posters to this report).

How were the results disseminated to communities of interest?
Abstracts were submitted to 2022 MHSRS - Military Health System Research Symposium with the title: "Targeted nutritional approach to improve muscle function and physical activity by restoring metabolic deregulations during recovery from sepsis: A study protocol of a pre-clinical trial". However, it was not accepted due to overwhelming amounts of manuscripts submitted to the symposium.(see appendices Abstract 3)

2022 Swine in Medical Research Conference. We submitted 3 abstracts with the titles: " Muscle Fatigue Measurements by Percutaneous Neural Stimulation in the Pig: Exploring a New Fatigue Protocol and Data Analysis.", "Progressive decrease of the BIA Phase Angle in a *Pseudomonas Aeruginosa* induced sepsis swine model. A marker of severity?", "Evaluation of Continuous Glucose Monitors in Septic Swine" These were accepted for three poster presentations. See appendices Abstract 2,4 and 5 and attached posters to this report.

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to report.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report.

What was the impact on other disciplines?
Nothing to report.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

CHANGES/PROBLEMS

Changes in approach and reasons for change.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them.
Due to university COVID-19 logistic restrictions, change in animal vendor and delay in animal supply, we have done less animals as indicated in the SOW. We expect to see improvement and some logistic adjustments in year 3 of this project to ensure the goals can be achieved.

Changes that had a significant impact on expenditures.
Due to university COVID-19 logistic restrictions, change in animal vendor and delay in animal supply, we have done less animals as indicated in the SOW.

Significant changes in use or care of human subjects.
Not applicable.

Significant changes in use or care of vertebrate animals.
Nothing to report.

Significant changes in use of biohazards, and/or select agents.
Nothing to report.

PRODUCTS

Publications, conference papers, and presentations.

Journal publications.
Nothing to report.

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers, and presentations.
Abstract were submitted to 2022 MHSRS - Military Health System Research Symposium with the title: "Targeted nutritional approach to improve muscle function and physical activity by restoring metabolic deregulations during recovery from sepsis: A study protocol of a pre-clinical trial". However, not accepted due to overwhelming amounts of manuscripts submitted to the symposium.

2022 Swine in Medical Research Conference. We submitted 3 abstracts with the titles: " Muscle Fatigue Measurements by Percutaneous Neural Stimulation in the Pig: Exploring a New Fatigue Protocol and Data Analysis.", "Progressive decrease of the BIA Phase Angle in a *Pseudomonas Aeruginosa* induced sepsis swine model. A marker of severity?", "Evaluation of Continuous Glucose Monitors in Septic Swine." These were accepted for three poster presentations. See appendices Abstract 2,4 and 5 and attached posters to this report.

2022 ESPEN conference (European Society for Clinical Nutrition and Metabolism) related to the present DOD project submissions (but not funded by DOD): with the titles: " Enhanced splanchnic extraction of nutritional arginine and lower citrulline production play a role in the reduced systemic availability of arginine in the early recovery phase of sepsis in the pig" (See appendices for abstract 1), and "Mainly muscle, jejunum, and lung fractional protein synthesis rates are related to whole-body protein synthesis in the pig" (See appendices for abstract 6). No approval known yet.

Website(s) or other Internet site(s).
Nothing to report.

Technologies or techniques.
Standardized and internationally recognized methods in rare disease, muscle function, and metabolism are used.

Inventions, patent applications, and/or licenses.
Nothing to report.

Other Products.
Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Nicolaas Deutz
Project Role:	PI
Research Identifier (e.g., ORCID ID):	0000-0001-5845-6447
Nearest person month worked:	1.92
Contribution to Project:	Responsible for all aspects of this study, including financial management, experimental design, stable tracer related methodological aspects.
Funding Support: Complete only if the funding support is provided from other than this award	DOD - HDTRA1-21-C-0006; NIH - 1R01HL132887-01A1; European Society for Clinical Nutrition and Metabolism, Internal

Name:	Gabriella Ten Have
Project Role:	Co-I
Research Identifier (e.g., ORCID ID):	0000-0003-2617-1193
Nearest person month worked:	6.60
Contribution to Project:	Responsible for design, implementing, coordinating and performing the animal experiments
Funding Support: Complete only if the funding support is provided from other than this award	DOD - HDTRA1-21-C-0006; NIH - 5R01DK120296; European Society for Clinical Nutrition and Metabolism, Internal

Name:	Marielle Engelen
Project Role:	Co-I
Research Identifier (e.g., ORCID ID):	0000-0001-9884-2553
Nearest person month worked:	1.20
Contribution to Project:	Responsible for coordination of overall aim of the pig study activities, mentoring research staff

Funding Support: Complete only if the funding support is provided from other than this award	DOD - HDTRA1-21-C-0006; NIH - 5R01AG064010-02; European Society for Clinical Nutrition and Metabolism, Internal

Name:	Peter Nghiem
Project Role:	Senior Personnel
Research Identifier (e.g., ORCID ID):	0000-0002-8796-8123
Nearest person month worked:	0.80
Contribution to Project:	Responsible for all measurements related to muscle function, endurance and strength, necropsy
Funding Support: Complete only if the funding support is provided from other than this award	Edgewise Therapeutics, R01EB028533, Fujifilm Diosynth biotechnologies, internal

Name:	John Thaden
Project Role:	Senior Personnel
Research Identifier (e.g., ORCID ID):	0000-0003-3381-2198
Nearest person month worked:	5.88
Contribution to Project:	Responsible for supervising and performing laboratory preparations and GC-MS/MS and LC-MS/MS analysis
Funding Support: Complete only if the funding support is provided from other than this award	DOD - HDTRA1-21-C-0006; Internal

Name:	Robert Beach
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	8.76
Contribution to Project:	Assisting with DOD experiments (blood processing, maknig nutrition intervention, general assistance), assisting with laboratory tasks, and prep work
Funding Support: Complete only if the funding support is provided from other than this award	DOD - HDTRA1-21-C-0006

Name:	Bailey Blair
Project Role:	Student Research Assistant

Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	1.80
Contribution to Project:	Assisting with DOD experiments (blood processing, maknig nutrition intervention, general assistance), assisting with laboratory tasks
Funding Support: Complete only if the funding support is provided from other than this award	Internal

Name:	Jiyeon Cha
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	9.72
Contribution to Project:	Assisting with DOD experiments (blood processing, maknig nutrition intervention, general assistance), assisting with laboratory tasks
Funding Support: Complete only if the funding support is provided from other than this award	DOD - HDTRA1-21-C-0006

Name:	Macie Mackey
Project Role:	Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	9.96
Contribution to Project:	Responsible for day-to-day coordination and performing animal experiments and laboratory analysis
Funding Support: Complete only if the funding support is provided from other than this award	DOD - HDTRA1-21-C-0006

Name:	Sarah Rice
Project Role:	Post-doc Research Associate
Research Identifier (e.g., ORCID ID):	0000-0002-5481-4558
Nearest person month worked:	8.52
Contribution to Project:	Responsible for day-to-day coordination and performing animal experiments and laboratory analysis

Funding Support: Complete only if the funding support is provided from other than this award	DOD - HDTRA1-21-C-0006; European Society for Clinical Nutrition and Metabolism

Name:	Alexis Rutledge
Project Role:	Research Staff
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	1.82
Contribution to Project:	Assistant to Co-I, Dr. Nghiem, for all measurements related to muscle function, endurance, and strength, necropsy
Funding Support: Complete only if the funding support is provided from other than this award	Edgewise Therapeutics, R01EB028533, internal

Name:	Elexa Smith
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	2.88
Contribution to Project:	Assisting with DOD experiments (blood processing, maknig nutrition intervention, general assistance), assisting with laboratory tasks
Funding Support: Complete only if the funding support is provided from other than this award	n/a

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to report.

SPECIAL REPORTING REQUIREMENTS

Quad Chart
Not applicable.

Award Chart

PR190829: Targeted Nutritional Approach to Improve Muscle Function and Physical Activity by Restoring Metabolic Deregulations During Recovery from Sepsis

PI: Nicolaas Deutz, Texas A&M University, Texas
Budget: $1,770,586
Topic Area: Peer Reviewed Medical Research Program
Mechanism: Investigator-Initiated Research

Research Area(s): SCS Coding
Award Status: 06/01/2020 – 05/31/2023

Study Goals: The major goal to the project is to test the hypothesis that a unique formulation based on EAA will improve physical activity, and involuntary isometric skeletal muscle strength faster during the recovery of a sepsis event.

Specific Aims: Our approach is to study the effects of targeted dietary EAA enriched nutritional supplementation versus a sham-control on muscle function and physical outcome in the recovery phase from sepsis (primary endpoint). Muscle function and physical outcome will be related to net protein synthesis on whole body and muscle level (secondary endpoint). In a randomized, sham controlled, blind (for nutritional intervention) 2 group design, we will use involuntary isometric skeletal muscle strength and physical activity measurements for functional outcome.

Key Accomplishments and Outcomes:
We performed studies in 32 animals with some delays due to COVID-19, change of animal vendor, and animal supply. We implemented/validated the analysis of the video's of the animal activity. An abstract was submitted to the 2022 Military Health System Research Symposium (not accepted) and three submitted to the Swine in Medical Research Conference (all accepted for poster presentations)
Publications: none to date
Patents: none to date
Funding Obtained: none to date

Abstract 1

Abstracts submitted to ESPEN 2022(European Society for Clinical Nutrition and Metabolism): related to the current DOD project, but not funded.

Title

Enhanced splanchnic extraction of nutritional arginine and lower citrulline production play a role in the reduced systemic availability of arginine in the early recovery phase of sepsis in the pig

Authors

Sarah A. Rice, Nicolaas E.P. Deutz, John J. Thaden, Marielle P.K.J. Engelen, Gabriella A.M Ten Have

Affiliation

Center for Translational Research in Aging & Longevity, Texas A&M University, College Station, TX, USA.

RATIONALE

Previously, we showed that essential amino acids (EAA) feeding, lacking non-essential amino acid (NEAAs), stimulates protein anabolism in the early recovery phase of sepsis in the pig. Because EAA does not contain arginine, a conditional essential amino acid in sepsis, we hypothesize that EAA feeding will stimulate the arginine *de novo* pathway from citrulline to arginine.

METHOD

In catheterized pigs, acute severe sepsis was induced for 6 hours (*Pseudomonas aeruginosa*). Post recovery, each pig received intra-gastric continuous feeding of a total amino acid (TAA, includes arginine, not citrulline) or EAA mixture (31 mg N/kg bw/h, dextrose 781 mg/kg bw/h) for 6 hours in 3 groups (Sham Healthy: H-TAA n=12 and Sepsis: S-TAA n=13, S-EAA n=12). Using stable isotope tracers 3 hours into feeding, metabolite whole body production (WBP) and concentrations were determined with LCMS/MS. Statistics: Data are mean [95%CI], α=0.05. Organ net release/uptake was analyzed with Wilcoxon. Group differences with AN(C)OVA.

RESULTS

As expected, arterial arginine concentrations are lower in S-TAA than H-TAA (p=0.0022) and lower in S-EAA compared to S-TAA (p=0.0037). Arginine is released more by the portal-drained viscera in S-TAA compared to S-EAA (p<0.0001), representing approximately 33% of arginine intake for S-TAA. However, all arginine is retained in the splanchnic area and no net arginine release was observed in both sepsis groups. In addition, citrulline WBP is lower in S-EAA compared to both S-TAA (p=0.0076) and H-TAA (p=0.0004). However, the lower availability of arginine did not stimulate *de novo* arginine WBP.

CONCLUSION

Here we confirm in the early phase of sepsis recovery the systemic availability of arginine is low, possibly related to the enhanced arginine splanchnic extraction and the reduced citrulline WBP production. We hypothesize that EAA feeding after sepsis needs to include arginine or citrulline to optimize its anabolic capacity.

	H-TAA	S-TAA	S-EAA
Arginine Concentration (μM)	73.1 [61.2,85.1]	53.06 [46.38,59.73]	33.61 [26.14,41.08]
Citrulline WBP (μmol/hr)	1225 [1086,1365]	1118 [945.6,1289]	826.8 [682, 971.5]
De novo Arginine WBP (μmol/hr)	849.6 [719.0, 980.2]	823.5 [645.8,1001]	770.3 [593.8, 946.8]

Abstract 2:

For 2022 Swine in Medical Research Conference

Title: (Title case)

Muscle Fatigue Measurements by Percutaneous Neural Stimulation in the Pig: Exploring a New Fatigue Protocol and Data Analysis.

Authors:

Gabriella A.M Ten Have[1], Peter P. Nghiem[2], Alexis M. Rutledge[2] Macie L. Mackey[1], Sarah A. Rice[1], Marielle P.K.J. Engelen[1], Nicolaas E.P. Deutz[1]

Affiliation:

[1] Center for Translational Research in Aging & Longevity, Texas A&M University, College Station, TX, USA.

[2]Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Introduction

Muscle fatigue is a major systemic feature in many chronic and acute diseases, negatively affecting daily functioning and health outcomes. Accurate assessment of muscle fatigue is therefore of critical importance. Muscle fatigue is defined as the decline in maximal force or power in time. Percutaneous maximal neural stimulation of the pelvic limb has been used to measure peak muscle strength in the dog and is viewed as a minimally-invasive technique that can be repeated over time. As no studies are available measuring muscle fatigue in the pig, we developed a new maximal neural stimulation protocol and evaluated this in the pig.

Methods

In anesthetized (I.V. ketamine/xylazine), healthy, catheterized pigs (n=15, ±25kg), muscle strength was measured isometrically (Aurora Scientific) in the right pelvic limb. Electrodes were placed around common peroneal nerves. The muscle fatigue exercise protocol consisted of thirty tetanus stimulations to induce tibiotarsal muscle FLEXIONS (fibular nerve) and EXTENSIONS (tibial nerve). Fatigue curves of the generated force or the maximum force per stimulation were fitted by a sigmoid decline curve (Graphpad Prism 9), to measure the area under the curves and obtain specific curve characteristics. Statistics: Data expressed as mean±SD. α=0.05. Correlations assessed by Spearman r.

Results

Total generated forces (corrected for body weight and 0.75% of foot length) were: FLEXION 7.2±4.6; EXTENSION 16±7 N-m/kg. Total force relative to fitted initial tetanus force (*RelForce)*: FLEXION 22±10; EXTENSION 22±11 %. Total decline (span of the curve, *Decline)* in force: FLEXION 72±25; EXTENSION 81±14 %. Fitted number of stimulations when 50% was declined (*Decline50)*: FLEXION 27±13; EXTENSION 17±4. Acceleration of the decline (hill slope) of force: FLEXION -5.1±7; EXTENSION -5.3±9. Limitation: The determination of the *Decline* in force was only possible in 65% of the curves due to a late onset of the decline or very slow decline. *Decline50* was correlated with *RelForce* ($p<0.0001$).

Conclusion:

It is feasible to assess muscle fatigue and related parameters in the pig by tetanus stimulations and subsequently fitting the decline curve in maximal generated force. However, the missing *Decline* values, particularly in the strong animals warrant a longer duration of the stimulation protocol.

Funding:
Department of Defense: CDMRP PR190829

Abstract 3:

For 2022 MHSRS - Military Health System Research Symposium

Targeted nutritional approach to improve muscle function and physical activity by restoring metabolic deregulations during recovery from sepsis: A study protocol of a pre-clinical trial.

Authors:

Gabriella A.M. Ten Have[1], Sarah A. Rice[1], Peter P. Nghiem[2], Macie L. McKay[1], John J. Thaden[1], Marielle P.K.J. Engelen[1], Nicolaas E.P. Deutz[1].

Affiliation:

[1] Center for Translational Research in Aging & Longevity, Department of Health & Kinesiology, Texas A&M University

[2] Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University

Introduction.

Infections and resulting sepsis have always been a significant problem on the battlefield. Sepsis is a potentially life-threatening complication of an infection in critically ill patients and characterized by severe tissue breakdown in several organs, leading to long-term muscle weakness, fatigue, and reduced physical activity. Sepsis continues to be the most expensive condition treated in acute care hospitals in the United States, and it has considerable cost for the Veterans Administration for both acute care and long-term care.

Our hypothesis is that early and targeted nutritional intervention is critical to enhance recovery and rehabilitation from sepsis. Our rationale is that developing novel nutritional approaches for critically ill patients will enhance recovery and rehabilitation by improved muscle mass and function. Use of translational animal models is essential to investigate the complex metabolic processes in relation to functional outcome. Therapeutic nutritional support in the pig model recovering from a septic event is viewed as highly translational to humans.

We recently used a multi-catheterized acute sepsis-recovery pig model to study in depth the protein kinetic disturbances in organs like gut, liver, and muscle and found o.a. accelerated whole body and muscle protein catabolism. We were also successful in increasing protein synthesis at the whole body level and in the muscle compartment in the first hours after sepsis by targeted nutritional supplementation with free essential amino acids (EAA). However, we were unable to identify if anabolic stimulation by EAA will result in an improved muscle function during the recovery of sepsis. Therefore, the following objectives for the current ongoing study are studied in depth in an instrumented clinical relevant pig model that is recovering from an acute septic infection of the Pseudomonas aeruginosa bacteria:

Specific Aim/Objective: to test the hypothesis that a unique formulation based on EAA will improve physical activity and involuntary isometric skeletal muscle strength during the recovery of a sepsis event.

Primary endpoint: we hypothesize that a unique formulation based on EAA will improve physical activity, and involuntary isometric skeletal muscle strength during the recovery of a sepsis event.

Secondary endpoint: we hypothesize that a unique formulation of EAA improves net muscle protein synthesis and muscle mass during the recovery of a sepsis event.

Materials and Methods.

Design. For both endpoints, we will use a clinically relevant non-lethal acute sepsis-recovery model. A chronically instrumented pig model (female Yorkshire cross/domestic pigs, ± 25 kg) prepared with indwelling catheters to enable sampling across the muscle compartment in a conscious state and to ensure a controlled administration of the nutritional intervention. After establishing a bacteremia induced acute septic state (*Pseudomonas aeruginosa*, 5 * 10^8 CFU/hour for 9 hours, I.V.), recovery will be initiated with antibiotics (gentamicin, 5 mg/kg, I.V.), and subsequent measurements of all endpoints will be started over a period of 7 days. On the first day after the septic event, nutritional intervention is started by bolus intragastric administration of one of the following meals: balanced free essential and non-essential amino acids (TAA; sham control) or essential amino acids (EAA). On day 1, 2 and 3 to 7 after start of the sepsis recovery phase, a total of 25, 50 and 100% respectively of daily nitrogen intake (0.56 gr N/kg bw of amino acid mixture; 15.4 gr/kg bw carbohydrates; 3.47 gr/kg bw crude fat; total 124 kcal/kg bw; vitamin-mineral mixture) will be administered intragastrically divided over 2 meals/day. The stepwise increase in the amount of enteral food administered will mimic the clinical occurrence of limited food intake in septic patients. Before (day -4) and after the start of the sepsis event (day 3 and 7), metabolic testing, muscle function, general activity, and clinical outcome will be measured to address the specific aim.

Metabolic testing. Catheters placed in blood vessels before and after the hindquarter allow us to determine the fluxes of amino acids, its catabolites and other food components across a muscle compartment and whole body level in a painless approach. Muscle protein breakdown and protein synthesis rates will be measured and compared between the TAA and EAA groups. The rate of organ protein synthesis, breakdown, and net protein balance will be calculated using a two-pool model. The model is based on measurements of arterial and venous concentrations and isotope enrichments of phenylalanine L-[^{15}N]-phenylalanine), and the rate of blood flow. This test will be performed under conscious post-absorptive and feeding conditions.

Muscle function is determined by an involuntary isometric skeletal muscle strength measurement under anesthesia. Current studies focus principally on the measurement of torque generated by the tibiotarsal joint. An analogous technique has been used to assess the strength of humans. Twitch and tibiotarsal joint flexion and extension torque is measured by a rapid-response servomotor/force transducer (model 310B LR, Aurora Scientific, Inc.) controlled by a PC using custom LabView software. The system is designed to enable physiology researchers to study the dynamic mechanical characteristics of muscle tissue.

The physical activity is measured with an established ethogram for video recording of the performed behavior of experimental pigs. General health status will be monitored by measurements of body temperature, general appearance, and body weight.

Statistics. Pigs (female Yorkshire cross/domestic pigs, ± 25 kg) will be randomized blindly to one of the 2 treatment groups: TAA, EAA. A total of 62 pigs (2 experimental groups) will be required to address all aims. This sample size is based on the assumption that 31 animals will be required in each group with an anticipated success rate of 80%, 31 animals per group will complete the primary endpoint (62 animals in total). Examination of the secondary endpoints will be implemented in the same animals. For the primary endpoint, we will score components of physical activity and muscle strength binomially. We compare the scores of two experimental groups with a Mann-Whitney test. For the secondary endpoints, we propose to compare groups using analysis of variance (ANOVA, ANCOVA) models. The following hypotheses of the primary endpoint concerning muscle function and physical activity. H0: TAA = EAA; H1: TAA ≠ EAA. The primary hypotheses will be tested at an unadjusted α-level of 5%.

Results

Milestones. Beside some delay due to COVID restrictions, we obtained the ethical approvals, implemented the logistics for monitoring physical activity and muscle strength measurement and other surgery/animal care/pharmaceutical compounding/ analytical related preparations. Currently we are conducting the animal

studies (17 pigs are completed). Preparations to set-up data analysis for the muscle strength measurements and automated physical activity measurement using tracking software for behavior video recordings have started. Laboratorium analyses of the specimen with mass spectrometry to study muscle metabolism are in place. For potential limitations, alternative strategies are implemented.

Conclusion.

The results of the currently ongoing pre-clinical study in a clinically relevant sepsis-recovery animal model will provide the basis for developing a new nutritional formulation to support muscle health in sepsis by stimulating protein synthesis. As part of our future translational studies, we will develop strategies to use this targeted nutritional approach in (veteran) septic patients to advance their clinical care and rehabilitation.

Abstract 4

For 2022 Swine in Medical Research Conference

Progressive decrease of the BIA Phase Angle in a *Pseudomonas Aeruginosa* induced sepsis swine model. A marker of severity?

Authors: S.A. Rice, G.A.M. Ten Have, M.L. Mackey, C.M. Van Sas, M.P.K.J. Engelen, NEP Deutz

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Background

Phase angle (PhA) is a strong indicator of cellular membrane integrity, is substantially decreased in critically ill patients and responds to nutritional intervention. We aimed to observe whether lower PhA are also present in a reversible sepsis model in pigs.

Methods

We induced acute, reversible sepsis in 21 conscious, catheterized pigs (Yorkshire, ±25 kg) for 9 hours via IV infusion of live *Pseudomonas Aeruginosa* and recovery induced by IV gentamycin. Heart rate (HR), systolic and diastolic blood pressure (BP), body temperature (BT) and phase angle (PhA: Bioimpedance Analysis (BIA,SFB7 Impedimed) were monitored until 6 hours after start recovery. Blood was analyzed for cytokines TNFα, IL-6 and IL-1β. Data are mean [95%CI], α=0.05. Data were lognormal transformed prior to statistics: Body weight, temperature, baseline values and hemodynamic parameters were used as confounders during ANCOVA analysis.

Results

HR, BP and BT increase during acute sepsis and resolve during post-sepsis recovery. Cytokines IL-6 ($p=0.004$), IL-1β ($p=0.005$) and TNFα ($p=0.003$) increase during acute sepsis. PhA decreases in relation to time during the acute septic event ($p<0.0071$, ANCOVA). Phase angle decreases from 11.55 [10.13, 12.98], directly prior to sepsis, to 9.789 [8.876, 10.70] after 9 hours of sepsis. Percent of phase decrease during sepsis was up to 22%, which is similar to the decrease seen in critically ill humans {Formenti, 2022 #1102}{Deutz, 2021 #834}. During recovery from sepsis, PhA measurements do not change in relation to time (ANCOVA).

Conclusion

Phase angle decreases during sepsis and accurately seems to relate to the progression of sepsis, but does not immediately resolve in early recovery. Our results indicate phase angle measurements can be used in swine models of sepsis. Overall, temporal decreases in PhA may signify shifts in body water status during the early progression of critical illness and recovery.

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Abstract 5

For 2022 Swine in Medical Research Conference

Title: Evaluation of Continuous Glucose Monitors in Septic Swine

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Background

The benefit of tight glucose control in the ICU is still a matter of intense debate. The FDA approved Continuous Glucose Monitors (CGM) for use in critically ill patients. Although a CGM measures glucose subcutaneously, it is developed for humans to estimate arterial blood glucose concentrations (40-500mg/dL) without the need to pre-calibrate with plasma/finger stick obtained concentrations. Unclear is if CGM can be used in a critical care swine model.

Methods

Female catheterized Yorkshire pigs (n=17, 22-27kg) had a CGM placed on the upper right abdominal quadrant (Abbott FreeStyle Libre Pro). Sepsis was induced in conscious pigs by i.v. infusion with live *Pseudomonas aeruginosa* to induce acute septicemia for 9-hours. Resuscitation with Ringers-Lactate and plasma glucose were controlled between 80-120mg/dL with 50% dextrose. Arterial plasma was sampled and measured every hour by Cobas c111.

Statistics

Glucose levels are expressed as mean±SD in mg/dL or mean [95% CI]. Accuracy: Paired T-test, Bland-Altman plots. Correlation: Pearson r. Precision: F-test. α=0.05.

Results

The CGM data below the minimum detection level of 3 pigs were excluded. Baseline values of CGM (n=14) in healthy pigs were lower compared to arterial values (CGM 66.9±11.7; arterial 74.1±3.5, p=0.045), and variation was higher in the CGM (p<0.0001). Through the entire septic event, CGM values were 8.0% [2.1%, 14.0%] lower (p<0.001) and had a weak correlation with arterial plasma values (n=137 glucose pairs, r^2=0.1691, p<0.0001). Also in the septic state, the variation of CGM was higher (p=0.0054).

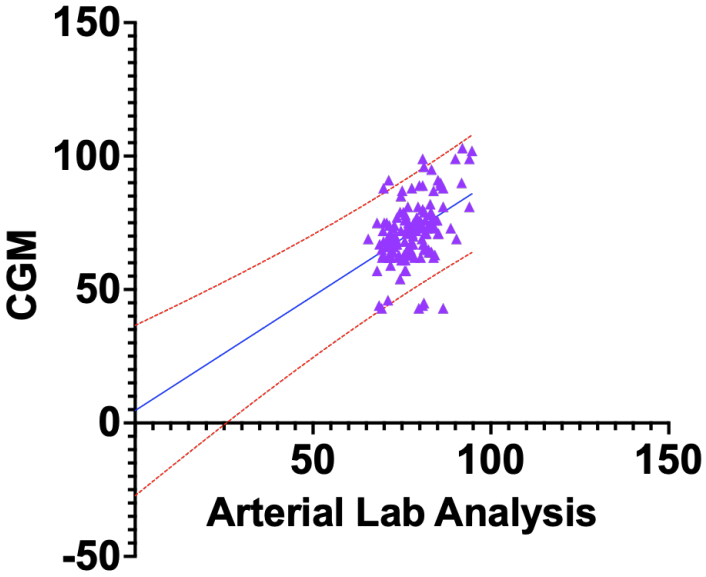
Conclusion

CGM only weakly reflects arterial glucose levels in healthy and septic pigs and has a much larger variation.

Funding:

Department of Defense: CDMRP PR190829

Correlation between Arterial Plasma and CGM



Abstract 6

Abstracts submitted to ESPEN 2022(European Society for Clinical Nutrition and Metabolism): related to the current DOD project, but not funded.

Title

Mainly muscle, jejunum, and lung fractional protein synthesis rates are related to whole-body protein synthesis in the pig

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Abstract

RATIONALE

To study protein metabolism, stable isotopes are used to measure whole-body protein synthesis (WbPS) and fractional protein synthesis rate (FSR) for tissues. In human studies, WbPS is often suggested to relate mainly to fractional muscle protein synthesis, but invasive tissue FSR measurements to confirm which organs contribute to WbPS are difficult. Therefore, we studied the relationship between WbPS and muscle FSR in the pig. Secondly, we studied WbPS and FSR in 11 other metabolic active organs.

METHODS

In 20 catheterized pigs (±25kg), A primed-continuous infusion of L-Phenylalanine (^{15}N-Phe) and Tyrosine (U-$^{13}C_9$-^{15}N-Tyr) stable isotopes was used for 8 hours. At steady-state, in the last three hours, blood was collected. Subsequently, the pig was euthanized and tissues were collected. Tracer enrichments in plasma and tissues were by LC-MS/MS. Statistics: Data are mean [95% CI]; α=0.05; WbPS in µmol/kg bw/hour. FSR in %/hour. Pearson correlation coefficient r, Primary outcome: WbPS versus Muscle FSR; Secondary: WbPS versus other tissues.

RESULTS

Muscle protein FSR was related to WbPS (r=0.451 [0.011,0.745], p=0.0458). Also jejunum and lung tissues were correlated with WbPS (jejunum: r=0.558 [0.153, 0.802], p=0.0106 and lung: r=0.512 [0.089, 0.778],p=0.021). Remarkably, other tissues showed no correlation.

CONCLUSION

Muscle, jejunum, and lung fractional protein synthesis rates are related to whole-body protein synthesis in the pig. Considering the muscle as the largest compartment in the body we hypothesize that whole-body protein synthesis reflects mainly muscle synthesis.