AWARD NUMBER: W81XWH-20-1-0259

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

REPORT DATE: July 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE

July 2023

2. REPORT TYPE

Annual

3. DATES COVERED

01Jun2022-31May2023

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER

W81XWH-20-1-0259

5b. GRANT NUMBER

12901301

5c. PROGRAM ELEMENT NUMBER

n/a

6. AUTHOR(S)

Nicolaas Deutz
Gabriella ten Have

E-Mail: nep.deutz@tamu.edu; gam.tenhave@tamu.edu

5d. PROJECT NUMBER

PR190829

5e. TASK NUMBER

n/a

5f. WORK UNIT NUMBER

n/a

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

Texas A&M University
Department of Kinesiology and
Sports Management
675 John Kimbrough Boulevard,
Suite 300
College Station, TX 77843-4243

8. PERFORMING ORGANIZATION REPORT NUMBER

n/a

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

PRMP

	11. SPONSOR/MONITOR'S REPORT NUMBER(S) n/a

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

n/a

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 3 Achievement: We performed studies in 54 animals, bringing the total number of animals for intention-to-treat analysis to 51. Preliminary results of the physical activity and muscle strength measurements show that ICU weakness related behavior changes and decline in muscle strength occur three days after the start of the sepsis-recovery.

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**
					USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	27	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

TABLE OF CONTENTS

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 a 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 3?

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 months 7-30. Planned milestone % completion at the end of year 3: 100%. We performed studies in 54 animals, bringing the total number of animals for intention-to-treat analysis to 51. Which reflects the goal to have at least 25 animals per experimental group. See flowchart. “Excluded” animals were animals with failures described for the potential 20% failure rate details of the Animal Use Protocol. No unexpected failures were identified.
Some delay was due to change/availability of personnel.

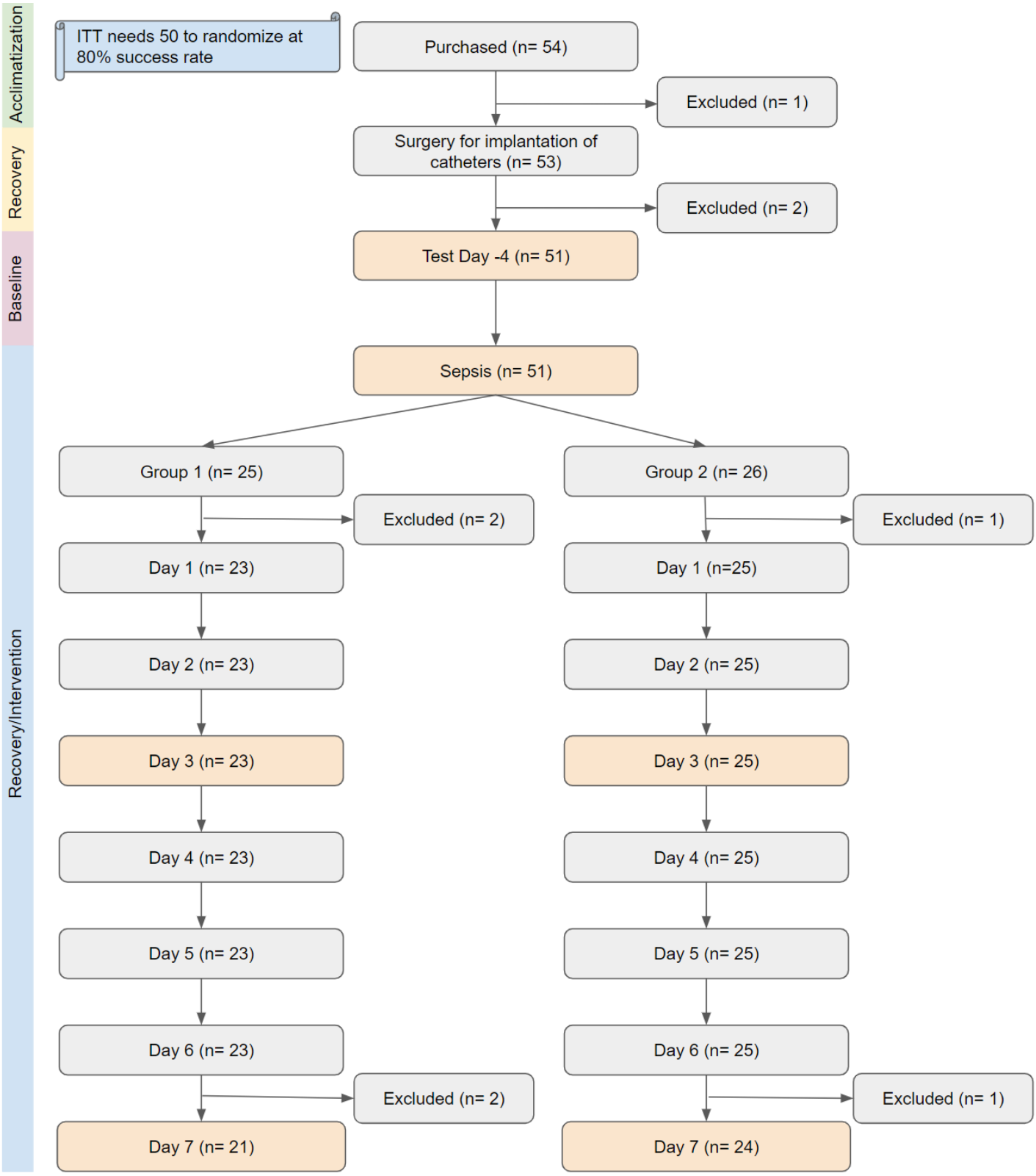


Figure 1: Flowchart

Major Task 2: Analyzing specimen - Activity (video) monitoring

Subtask 1: For metabolic analysis. Analysis specimen with Mass Spectrometry. Planned between months 12-30. Planned milestone % completion at the end of year 3: 100%. Current % of completion: 15%: Getting mass spectrometry applications validated and in place. It resulted in the submission of abstracts of previous research in an animal model related to the used animal model and metabolic analysis in the present

DOD-project (Title 1: 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. Title 2: Mainly muscle, jejunum, and lung fractional protein synthesis rates are related to whole-body protein synthesis in the pig). **See appendices for published Abstract 1&2**. Abstracts were presented at the ESPEN 2022 conference (European Society for Clinical Nutrition and Metabolism). **See Poster 1 in the appendices**. Batch wise specimen analysis will start in year 4. Some delay was due to delay in major task 1, subtask 3

Subtask 2: Analysis of the video of the animal activity. Planned between months 12-30. Planned milestone % completion at the end of year 3: 100%. Current milestone % of completion 50%: We implemented/validated the analysis of the video's of the animal activity. Assessing physical activity an automatic video tracking system (Ethovision, Noldus) with the developed ethogram which contained selected behaviors related to ICU weakness (**see Table 1**) was performed with daytime videos of 22 animals. We established that in the present project, behavior related to ICU weakness could be detected see **Figure 2.** Analyses of the rest of the videos will be performed in year 4. Some delay was due to delay in major task1, subtask 3.

Table 1: Ethogram (home cage measurements)
Goal: To detect potential development of behavior symptoms related to ICU weakness in a sepsis recovery pig model: Fatigue, Depression

ICU weakness related Behavior	**Measured as**	**Measured by**
Less active	Percentage of activity in the home cage	Video pixel changes
	Distance moved	Tracking centerpoint of the animal
	Duration of movement	Tracking centerpoint of the animal
Lower gait speed	Maximum velocity of the animal	Tracking centerpoint of the animal
Harder to start moving	Maximum acceleration of the animal	Tracking centerpoint of the animal
Less interaction with cage enrichments	Interaction with Triangle	Activity (video pixel changes) in the triangle area
	Rooting behavior: kicking a ball: maximum acceleration of ball	Tracking centerpoint of the ball
	Duration of ball movement	Tracking centerpoint of the ball
Less social interaction with other animals	Resting in area alongside another area with another animal	Tracking centerpoint and no pixel changes (non-activity) in the social area

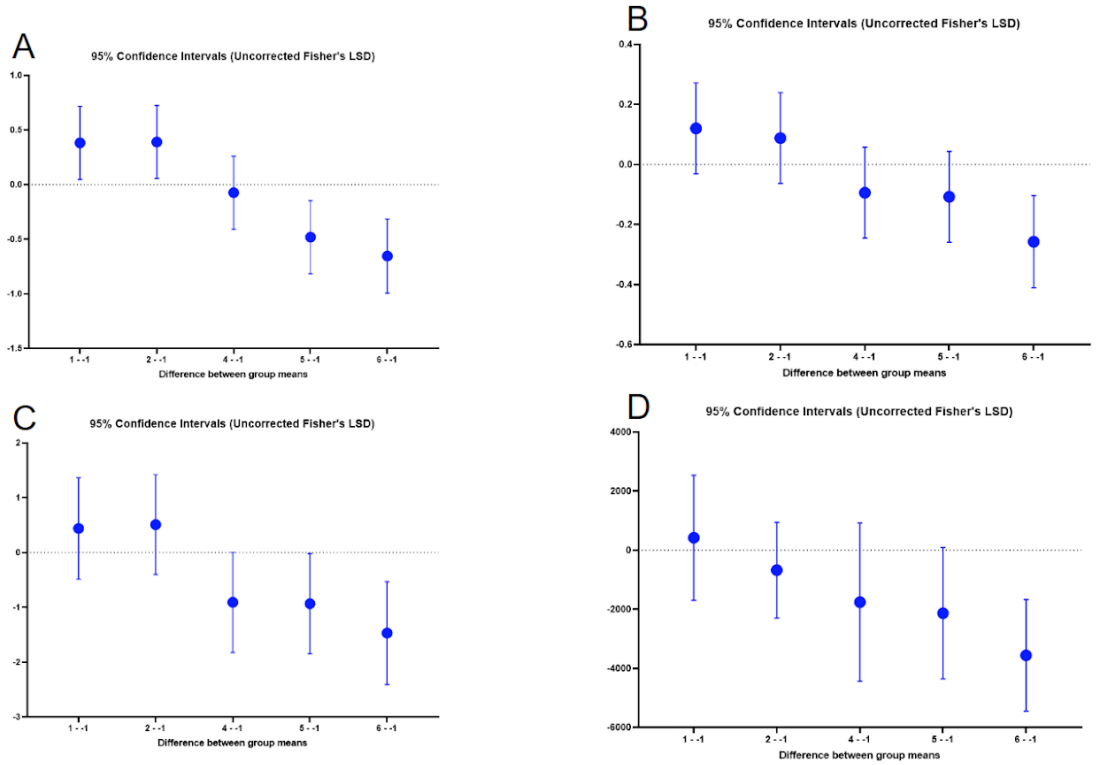


Figure 2: Preliminary results. ICU weakness related behavior.

A: Activity, B: Maximum gait speed, C: Rooting behavior, D: Social Interaction. Behavior of sepsis-recovery day 1,2,4,5 and day 6, between 11:00 and 14:00 were compared with baseline day (day-1). Data of figure A,B and C were log transformed. One-Way mixed effect analysis. N= 22

Subtask 3: Analysis of the muscle strength responses. Planned between months 12-30. Planned milestone % completion at the end of year 3: 100%. Current milestone % of completion 50%: Implemented the analysis of the muscle strength responses was performed at the end of year 3. We could establish with preliminary results of 31 animals that in the present project, muscle strength weakness in the recovery period could be detected. Before (day -4, baseline) and after the start of the sepsis event (day 3 and 7), involuntary, isometric skeletal muscle strength was measured as a marker of muscle function under anesthesia. After baseline twitch and tetanus was performed, a muscle fatigue exercise protocol was performed, consisting of 30-40 consecutive tetanus stimulations. Muscle function did not change as reflected by comparable values for maximum force of the tibiotarsal muscle flexion. However, the flexor muscles did fatigue more rapidly (steeper downslope of the fatigue curve) on recovery day 3 and 7 **(Figure 3).** Analyses of the rest of the muscle strength responses will be performed in year 4. Some delay was due to delay in major task 1, subtask 3.

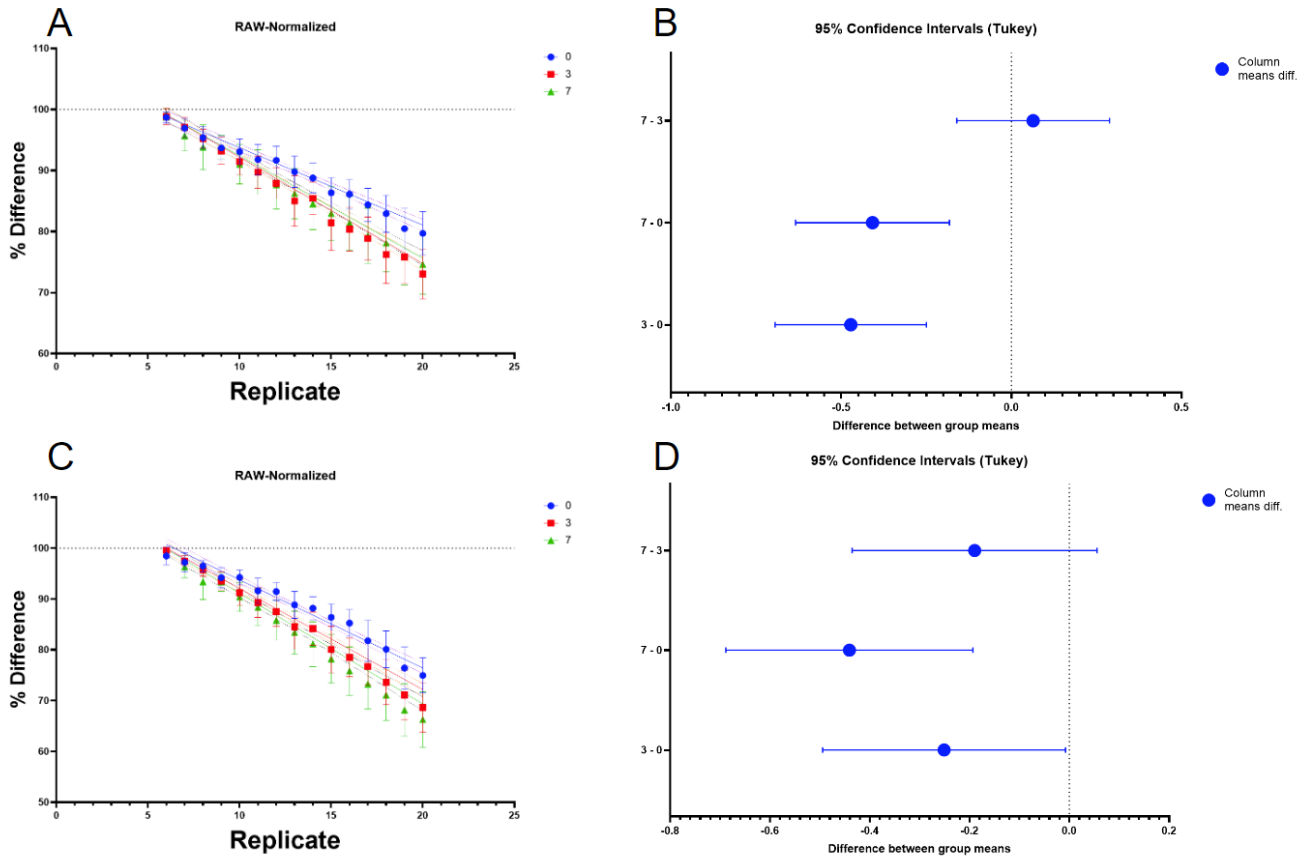


Figure 3. Preliminary results, muscle strength. A muscle fatigue exercise protocol was performed, consisting of 30-40 consecutive tetanus stimulations **A.** Fatigue slopes of flexor muscles, tetanus peaks. **B.** Slope comparison with baseline measurement, tetanus peaks **C.** Fatigue slopes of flexor muscles, tetanus area under the curve, **D.** Slope comparison with baseline measurement, tetanus area under the curve. Data were normalized to maximum force between tetanus #6-20. N=31 animals. Slopes were compared with baseline measurements with one-way ANOVA.

Major Task 3: Data modeling - statistical data analysis

Subtask 1: Data modeling - statistical data analysis: Planned between month 31-36. Current Milestone completion 20%. With preliminary data analysis in year 3, we could establish that in the present project, behavior/muscle strength changes related to ICU weakness could be detected. See above **Figure 2 & 3**. This resulted in the submission of an abstract to 2023 MHSRS - Military Health System Research Symposium with the title: "An ICU weakness translational pig model: Characteristics of compromised muscle function and physical activity during recovery of a Pseudomonas aeruginosa induced septic event." (**Abstract 3**) and ESPEN 2023 conference (Title: Compromised muscle function and physical activity during recovery of sepsis in an ICU-Acquired Weakness translational pig model (**Abstract 4**). This subtask will be completed in year 4. Some delay was due to delay in major task1, subtask 3.

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 continued in year 3: 2023 MHSRS - Military Health System Research Symposium, "An ICU weakness translational pig model: Characteristics of compromised muscle function and physical activity during recovery of a Pseudomonas aeruginosa induced septic event." However, this abstract was not accepted for poster presentation due to an overwhelming amount of abstracts submitted to the symposium. See **Abstract 3** in appendices.

We presented abstracts for the 2022 ESPEN conference 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**, poster presentation), and "Mainly muscle, jejunum, and lung fractional protein synthesis rates are related to whole-body protein synthesis in the pig" (See appendices for **Abstract 2**, oral presentation (best abstract session)).

We submitted for the 2023 ESPEN conference related to the present to the DOD project with the titles: "Compromised muscle function and physical activity during recovery of sepsis in an ICU-Acquired Weakness translational pig model" (**Abstract 4**). "Does whole body measured splanchnic extraction of amino acids with stable isotopes tracers in the prandial state represent tracee splanchnic extraction?" (**Abstract 5**). Both are accepted for the 11th-14th September 2023 conference as poster and oral presentation, respectively.

What opportunities for training and professional development has the project provided?
In the third year, Dr. Sarah Rice left the research group. Ms. Mackey was trained and developed professional skills to take over the practical day-to-day coordination tasks of the pig studies (previously done by Dr. Rice). Additional staff (Dr. Simbo) was trained in the practical care and documentation needed to house the animals and ensure their well-being throughout the course of the study and he developed professional skills from formal surgical prep, maintenance of chronic catheters, animal care during critical illness and recovery and performing experiments. Also, Ms. Carolina Perez was hired as staff and trained by Dr. Thaden in the chemical analytical laboratory to assist in the performing of the mass spectrometry analysis. Mr. Argyelan was onboarded to assist and received the opportunity to get experience with various aspects of preparation/ processing of the samples, and data entry. Ruebush also assisted with logistics of supplies, data entry, and audit. The student research assistants (Mr. Beach, Ms. Cha, Mr. Daasari, Ms. George, Ms. Palmiere, Ms. Poates, Ms. Russell) have developed skills to assist in preparation and performing complex animal studies.

Dr. Ten Have translated an ICU weakness ethogram into a tracking protocol with an automatic video tracking system (Ethovision, Noldus). Drs. Deutz and Ten Have set-up the data analyses of these video tracking

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.

Drs. Ten Have and Rice had the opportunity to present/discuss scientific results related to the present DOD project with experts in the field during the 2022 ESPEN Conference (see appendices **Abstract 1 and 2, and Poster 1**)

How were the results disseminated to communities of interest?
In year 3, we presented abstracts for the 2022 ESPEN conference 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**, poster presentation), and "Mainly muscle, jejunum, and lung fractional protein synthesis rates are related to whole-body protein synthesis in the pig" (See appendices for **Abstract 2**, oral presentation (best abstract session)).

Abstract were submitted to 2023 MHSRS - Military Health System Research Symposium with the title: 2023 MHSRS - Military Health System Research Symposium with the title: "An ICU weakness translational pig model: Characteristics of compromised muscle function and physical activity during recovery of a Pseudomonas aeruginosa induced septic event."However, it was not accepted due to overwhelming amounts of manuscripts submitted to the symposium.(see appendices **Abstract 3**)

We submitted for the 2023 ESPEN conference related to the present to the DOD project with the titles: "Compromised muscle function and physical activity during recovery of sepsis in an ICU-Acquired Weakness translational pig model" (**Abstract 4**). "Does whole body measured splanchnic extraction of amino acids with

stable isotopes tracers in the prandial state represent tracee splanchnic extraction?" (**Abstract 5**). Both are accepted for the 11th-14th September 2023 conference as a poster and oral presentation, respectively.

What do you plan to do during the next reporting period to accomplish the goals?
Final analysis of samples and statistical modeling will be completed during the next performance period. We anticipate to present 2 abstracts (ESPEN 2023) and submit 2 more abstracts to (inter) national conferences, and 2 manuscripts to peer-review journals with high impact factors in the field.

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.
We completed all the animals by the end of Year 3. During the no-cost-extensions period, we will finalize data/sample analysis, final report, and manuscript(s).

Changes that had a significant impact on expenditures.
Nothing to report.

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.

Ten Have G.A.M., Thaden J.J., Engelen M.P.K.J., Deutz N.E.P. Muscle, jejunum and lung fractional protein synthesis are prominently correlated with whole body protein synthesis in the pig. 2022 European Society for Parenteral and Enteral Nutrition. published: Clinical Nutrition ESPEN, vol 54, page 469, 10.1016/j.clnesp.2022.09.044

Rice S.A., Deutz N.E.P., Thaden J.J., Engelen M.P.K.J., Deutz N.E.P., Ten Have G.A.M. 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. 2022 European Society for Parenteral and Enteral Nutrition. Published: Clinical Nutrition ESPEN, Vol 54, Page 496, DOI: 10.1016/j.clnesp.2022.09.117

Abstract were submitted to 2023 MHSRS - Military Health System Research Symposium with the title: 2023 MHSRS - Military Health System Research Symposium with the title: “An ICU weakness translational pig model: Characteristics of compromised muscle function and physical activity during recovery of a Pseudomonas aeruginosa induced septic event.”However, it was not accepted due to overwhelming amounts of manuscripts submitted to the symposium (see appendices **Abstract 3**).

We submitted for the 2023 ESPEN conference (European Society for Clinical Nutrition and Metabolism) related to the present to the DOD project with the titles: “Compromised muscle function and physical activity during recovery of sepsis in an ICU-Acquired Weakness translational pig model” (**Abstract 4**). “Does whole body measured splanchnic extraction of amino acids with stable isotopes tracers in the prandial state represent tracee splanchnic extraction?” (**Abstract 5**). Both are accepted for the 11th-14th September 2023 conference as resp. a poster and oral presentation.

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:	0.60
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	DOD - HDTRA1-21-C-0006; European Society for Clinical Nutrition and Metabolism, Internal

than this award	

Name:	Gabriella Ten Have
Project Role:	Co-I
Research Identifier (e.g., ORCID ID):	0000-0003-2617-1193
Nearest person month worked:	4.08
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:	0.84
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:	0.11
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	DoD W81XWH-22-1-0988, NIH 1-R01AR08129, NIH 5-R01EB028533-03, FujiFilm collaboration 2110122

Name:	John Thaden
Project Role:	Senior Personnel
Research Identifier (e.g., ORCID ID):	0000-0003-3381-2198
Nearest person month worked:	4.80
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:	David Argyelan
Project Role:	Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	1.20
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; NIH - 5R01AG064010-02; Internal

Name:	Robert Beach
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	1.20
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; University of Arkansas for Medical Science; American Society for Parenteral and Enteral Nutrition

Name:	Jiyeon Cha
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	0.96
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; University of Arkansas for Medical Science; American Society for Parenteral and Enteral Nutrition

Name:	Yashdeep Daasari
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	1.08
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; University of Arkansas for Medical Science; American Society for Parenteral and Enteral Nutrition
Name:	Eliza George
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	1.20
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:	Macie Mackey
Project Role:	Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	8.64
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:	Alice Palmiere
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	1.20

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	none

Name:	Carolina Perez
Project Role:	Research Staff
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	6.48
Contribution to Project:	Responsible for 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:	Catherine Poates
Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	0.45
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; University of Arkansas for Medical Science; American Society for Parenteral and Enteral Nutrition; European Society for Clinical and Nutrition Metabolism

Name:	Laura Ruebush
Project Role:	Research Staff
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	0.72
Contribution to Project:	Monitors logistics, audits data for reporting
Funding Support: Complete only if the funding support is provided from other than this award	NIH - 5R01AG064010-02; NIH - 5R01DK120296; NSF - 1648451; Internal

Name:	Haley Russell

Project Role:	Student Research Assistant
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	2.20
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	none

Name:	Alexis Rutledge
Project Role:	Research Staff
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	0.06
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	NIH 1-R01AR08129, NIH 5-R01EB028533-03

Name:	Sunday Simbo
Project Role:	Research Staff
Research Identifier (e.g., ORCID ID):	n/a
Nearest person month worked:	2.40
Contribution to Project:	
Funding Support: Complete only if the funding support is provided from other than this award	Responsible for day-to-day coordination and performing animal experiments and laboratory analysis

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 (ESPEN 2022 conference)

Clinical Nutrition ESPEN
ABSTRACT| VOLUME 54, P496, APRIL 2023
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
S.A. Rice
N.E. Deutz
J.J. Thaden
M.P. Engelen
G.A. Ten Have
DOI:https://doi.org/10.1016/j.clnesp.2022.09.117

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]

Poster 1

44th ESPEN CONGRESS

3–6 Sept 2022
VIENNA ESPEN CONGRESS
ON CLINICAL NUTRITION & METABOLISM
www.espen.org www.espencongress.com

eat & move

Jointly Organised with Austria Croatia Slovenia

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, S. RICE, N.E.P DEUTZ, J. THADEN, M.P.K.J. ENGELEN, G.A.M. TEN HAVE*
Center for Translational Research in Aging & Longevity. Texas A&M University, United States of America
*Presenting Author

PT19

INTRODUCTION

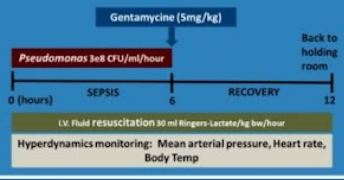
Previously, we showed that essential amino acids (EAA) feeding, lacking non-essential amino acid, stimulates protein anabolism in the early recovery phase of sepsis in the pig. Because EAA does not contain arginine, a conditionally essential amino acid in sepsis, we hypothesize that EAA feeding will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

AIM

Determine how EAA feeding influences transorgan Arginine (Arg) – Citrulline (Cit) net fluxes and whole body *de novo* Arginine and Citrulline production (WBP) in early sepsis recovery

MODEL

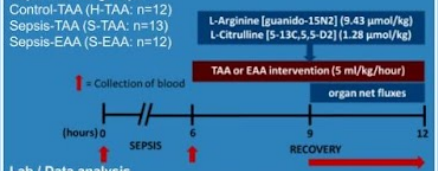
Pseudomonas aeruginosa acute sepsis-recovery model:
Female Catheterized pig (± 25 kg)



STUDY DESIGN

Intervention:
TAA = Total amino acid (AA) mixture with a pig muscle AA profile.
EAA = Essential amino acid mixture with a pig muscle EAA profile
All mixtures were iso-nitrogen (0.031gr N/kg bw/h), iso-caloric (0.78 gr/kg bw/h of maltodextrin), iso-ionic (for K, Ca, Mg and Na), administered intra-gastric for 6 hours (30% daily intake)

Experimental groups:
Control-TAA (H-TAA: n=12)
Sepsis-TAA (S-TAA: n=13)
Sepsis-EAA (S-EAA: n=12)



Lab / Data analysis
- Amino acid concentrations and tracer enrichments: LC/MS/MS
- Plasmaflow by para-aminohippuric acid dilution
- Net flux = organ plasma flow x ([Conc]arterial – [Conc]venous)
- Whole Body Production (WBP) as described in Engelen M. et al. Curr Opin Clin Nutr Metab Care 2019 Vol. 22 Issue 5 Pages 337-346

Statistics: Data are mean [95%CI], α=0.05. Organ net release/uptake was analyzed with Wilcoxon Signed Rank test. Group differences with AN(C)OVA. Significance: p<0.05

RESULTS

Systemic Arginine Availability is Low in Early Sepsis Recovery, Specifically EAA-fed Animals

	Healthy-TAA	Sepsis-TAA	Sepsis-EAA
Arginine	72.94 [62.1,83.7]	53.06** [46.4,59.7]	33.61** [26.1,41.1]
Citrulline	48.6 [41.7,55.5]	38.6* [32.8,44.4]	36.9** [34.0,39.8]

Table 1. Arterial Arginine and Citrulline concentrations (in µM) *ANOVA post-hoc Tukey, H-TAA vs. S-TAA and S-TAA vs. S-EAA,** signifies p<0.005, * signifies p<0.05*

Splanchnic Extraction of Arginine is enhanced in Early Sepsis Recovery Compared to Healthy Animals

ARGININE	Healthy-TAA	Sepsis-TAA	Sepsis-EAA
Splanchnic Region	-804.9* [-1110,-500.1]	-62.9 [-265.2,138.4]	78.8 [-95.81,253.4]
Portal Drained Viscera (PDV)	-735.9* [-959.9,-511.8]	-404.9* [-673.7,-136.0]	-66.0* [-107.5,-24.5]
Kidneys	-203.4 [-454.5,47.6]	-109.0 [-256.4,38.3]	-103.5* [-166.3,-40.6]

Table 2. Arginine organ net fluxes (in Splanchnic Region, Portal Drained Viscera, and Kidney). *Splanchnic extraction is higher in Healthy animals than either sepsis group (p<0.001, one-way ANOVA, post-hoc Tukey). Net Flux positive values represent uptake by the organ, negative values represent release. *) Different from zero. Data shown is nmol/kg bw/min.*

CITRULLINE	Healthy-TAA	Sepsis-TAA	Sepsis-EAA
Splanchnic Region	-581.5* [-739.3,-423.7]	-360.9* [-534.5,-187.3]	-355.6* [-415.6,-295.6]
Portal Drained Viscera (PDV)	-413.4* [-513.4, -313.4]	-322.3* [-408.2,-236.5]	-260.5* [-342.2,-178.8]
Kidneys	423.3* [193.3, 653.2]	223.0* [144.3,301.6]	308.9* [209.5,408.3]

Table 3. Citrulline organ net fluxes (in Splanchnic Region, Portal Drained Viscera, and Kidney). *Net Flux positive values represent uptake by the organ, negative values represent release. *) Different from zero. Data shown is nmol/kg bw/min.*

EAA-fed Sepsis Recovery Animals have lower citrulline production than TAA-Sepsis and Healthy animals, but do not increase *de novo* Arginine Production

Fig. 1

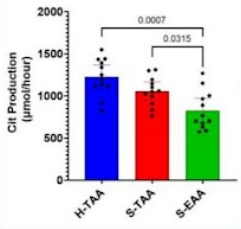


Figure.1 WBP of Citrulline. *Data were LN transformed. ANCOVA with body weight, mean arterial pressure and heart rate as a co-variants.*

Fig. 2

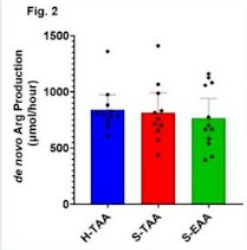


Figure.2 de novo Arginine production *Lower arginine availability did not stimulate de novo arginine WBP synthesis. Data were LN transformed. ANCOVA with weight, mean arterial pressure and heart rate as co-variants.*

CONCLUSIONS

- Here we confirm in the early phase of sepsis recovery, the systemic availability of arginine is low. This is related to the enhanced arginine splanchnic extraction and the reduced citrulline WBP production.
- In contrast with EAA stimulated anabolic protein response, EAA did not stimulate *de novo* Arginine synthesis.
- We hypothesize that EAA feeding after sepsis needs to include arginine or citrulline to optimize its anabolic capacity.

ACKNOWLEDEMENT

CTRAL Staff, Interns and veterinary staff

CONTACT INFORMATION

gam.tenhave@ctral.org
s.rice@ctral.org

TEXAS A&M UNIVERSITY
Center for Translational Research in Aging and Longevity

FUNDING

NIH R01GM084447
S10RR027047
Texas A&M College Award - 2020 Catapult

Abstract 2 (ESPEN 2022 conference):

Clinical Nutrition ESPEN
ABSTRACT| VOLUME 54, P469, APRIL 2023
Mainly Muscle, Jejunum, And Lung Fractional Protein Synthesis Rates Are Related To Whole-Body Protein Synthesis In The Pig
G. A. Ten Have
J.J. Thaden
M.P. Engelen
N.E. Deutz
DOI:https://doi.org/10.1016/j.clnesp.2022.09.044

ORAL PRESENTATION (best abstract session)

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 (15N-Phe) and Tyrosine (U-13C9-15N-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.

Abstract 3 (2023 MHSRS conference)

Abstract ID: MHSRS-23-10185

Title

An ICU weakness translational pig model: Characteristics of compromised muscle function and physical activity during recovery of a *Pseudomonas aeruginosa* induced septic event.

Authors

Gabriella A.M. Ten Have PhD[1], Peter P. Nghiem PhD, DVM [2], Macie L. Mackey BSc[1], Celine van Sas BSc[1], Sarah A. Rice PhD[1], Marielle P.K.J. Engelen PhD[1], Nicolaas E.P. Deutz PhD, MD[1].

Affiliation

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

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

Abstract

Introduction

Infections and resulting sepsis have always been a significant problem on the battlefield. Sepsis is a potentially life-threatening complication in critically ill patients and characterized by severe tissue breakdown in several organs, leading to long-term muscle weakness, fatigue, and reduced physical activity (ICU-Acquired Weakness=ICU-AW). 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 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 improving muscle mass and function. Use of translational large 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 developed 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 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. However, we were unable to identify if anabolic stimulation by essential amino acids will result in an improved muscle function during the recovery of sepsis. Therefore in the current ongoing study, we are studying in depth muscle function in an instrumented clinical relevant pig model that is recovering from an acute septic infection of the *Pseudomonas aeruginosa* bacteria. Although the study is still ongoing, we could characterize the muscle strength and the physical activity changes due to the acute septic event in a subset of animals by measuring involuntary isometric skeletal muscle strength under anesthesia and video tracked spontaneous homecage physical activity.

Materials and method

We used a clinically relevant, non-lethal, acute sepsis-recovery model. We used a chronically instrumented pig model (female Yorkshire cross/domestic pigs, ± 25 kg) with pre-implanted indwelling catheters that

enables sampling across the muscle compartment in the conscious state and that ensures 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 was initiated with antibiotics (gentamicin, 5 mg/kg, I.V.), and subsequent measurements of all endpoints were started over a period of 7 days. On the first day after the septic event, nutritional intervention was started by bolus intragastric administration. 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) were administered intragastrically divided over 2 meals/day. The stepwise increase in the amount of enteral food administered mimicked the clinical occurrence of limited food intake in septic patients. General health status was monitored by measurements of body temperature, general appearance, and body weight.

Muscle function. Before (day -4, baseline) and after the start of the sepsis event (day 3 and 7), involuntary, isometric skeletal muscle strength was measured as a marker of muscle function under anesthesia. Current studies focus principally on the measurement of torque generated by the flexor and extensor muscles of the tibiotarsal joint (TTJ). An analogous technique has been used to assess the strength of humans. Twitch and tetanic torque was measured by a rapid-response servomotor/force transducer (model 310B LR, Aurora Scientific, Inc.) controlled by a PC using custom LabView software. The system was designed to study the dynamic mechanical characteristics of muscle tissue. In the anesthetized (I.V. ketamine/xylazine) animals, electrodes were placed in the right pelvic limb around the fibular/common peroneal nerve and the tibial nerve to stimulate flexion or extension of the TTJ muscles, respectively. After baseline twitch and tetanus was performed, a muscle fatigue exercise protocol was performed, consisting of 30-40 consecutive tetanus stimulations.

Homecage physical activity. Physical activity was measured by tracking the animals 24/7 by video recording and tracking software (Noldus, Ethovision XT17) using an ethogram reflecting animal behaviors that would relate to ICU weakness like muscle weakness, fatigue, and depression (less active, lower gait speed, harder to start walking, less interaction with cage enrichments). In the present characterization, we compared the daily videos (between 11am and 2pm) obtained at day -1 (Baseline) and the recovery days 1, 2, 4, 5, and 6.

Statistics.
Statistics were performed with Graphpad Prism 9.5. The distribution of data was determined and when needed, data were log transformed before further analyses.
Muscle function: Average maximum force and downslopes of the fatigue curves of the baseline measurement (day -4) were compared with the measurements of recovery days 3 and 7 (one-way ANOVA).
Homecage physical activity: Baseline (Day-1) behaviors were compared with day 1, 2, 4, 5, and 6 using a mixed effect analysis. Post-hoc analysis was done with an uncorrected Fisher's LSD. The data reflected an interim analysis of an ongoing study. $p<0.05$ was considered statistically significant.

Results
Muscle function (N=31). Muscle function did not change as reflected by comparable values for maximum force of the tibiotarsal muscle flexion. However, the flexor muscles did fatigue more rapidly (steeper downslope of the fatigue curve) on recovery day 3 ($p=0.0415$) and 7 ($p<0.0001$).
Homecage physical activity (N=22). General activity was increased in the first 2 recovery days when food was restricted (day 1: $p=0.0257$, day 2: $p=0.0228$), but decreased below baseline thereafter (day 5: $p=0.0053$, day 6: $p=0.0002$). Maximum gait speed was lower on recovery day 6 ($p=0.0012$). It was harder for the animals to start walking (maximum acceleration to move) on day 6 ($p=0.0091$). Interaction with cage enrichments: rooting behavior (interaction with ball) was less forceful on days 5 ($p=0.094$) and 6 ($p=0.0002$). A biting ring was used less on day 5 ($p=0.0074$) and 6 ($p=0.0298$).

Conclusion
Our chronic instrumented sepsis-recovery model in the pig showed muscle fatigue and altered physical behavior that can be related to ICU weakness. Therefore this model can be considered as translatable to humans and clinically and scientifically important to advance ICU-AW research.

The results of the currently ongoing preclinical 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 research line, we will develop strategies to use this targeted nutritional approach in septic patients (i.e. injured military personnel) to advance their clinical care and rehabilitation.

Abstract Disclaimer
Nothing to disclaim

Learning Objectives:

1. Describe an acute septic-recovery model in a clinical relevant animal model.
2. Describe a clinical relevant ICU-Aquired Weakness animal model to study new nutritional strategies to support muscle health.
3. Describe a combination of techniques to study direct relations between muscle metabolism and muscle function.

Abstract 4 (2023 ESPEN)

Topic: Critical care

Abstract Submission Identifier: ESPEN23-ABS-2018

Title

Compromised muscle function and physical activity during recovery of sepsis in an ICU-Acquired Weakness translational pig model.

Authors

Gabriella A.M. Ten Have PhD1, Peter P. Nghiem PhD, DVM 2, Macie L. Mackey BSc1, Celine van Sas BSc1, Sarah A. Rice PhD1, Marielle P.K.J. Engelen PhD1, Nicolaas E.P. Deutz MD, PhD1.

Affiliation

1Center for Translational Research in Aging & Longevity, Texas A&M University

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

Rationale. Sepsis is characterized by severe tissue breakdown in several organs, leading to long-term muscle weakness, fatigue, and reduced physical activity (ICU-Acquired Weakness). Use of a translational large animal model is essential to investigate the complex metabolic processes underlying compromised functional performance during recovery from sepsis. Therefore we studied skeletal muscle function in an instrumented clinical relevant Pseudomonas induced sepsis pig model by measuring muscle strength/ fatique, and home cage physical activity.

Methods. In a 7-day long sepsis-recovery model in 31 pigs (± 25 kg), involuntary, isometric skeletal muscle strength was measured under anesthesia in the right pelvic limb on day -4 (baseline), 3 and 7. Peak force and downslopes of fatigue curves were obtained from tetanus stimulations of the tibiotarsal muscle flexors (fibular nerve) and extensors (tibial nerve). Physical activity (N=22) was obtained with video tracking software (Noldus, Ethovision XT17) using an ethogram reflecting ICU weakness behaviors. Statistics: Comparison with baseline. ANOVA. α=0.05.

Results. The flexor muscles showed a steeper downslope in the fatigue curve on day 3 (p=0.0415) and 7 (p<0.0001). No change in peak strength. On day 5 and 6 general physical activity was decreased (p=0.0053, p=0.0002). On day 6, maximum gait speed and acceleration to start walking was lower(p=0.0012, p=0.0091). On day 5 and 6, the interaction with cage enrichments was less: rooting behavior (p=0.094, p=0.0002), biting ring (p=0.0074, p=0.0298).

Conclusions. Our sepsis-recovery model in the pig is able to reflect the most important functional characteristics of human ICU-Acquired Weakness (muscle fatigue, and altered physical behavior) and therefore can be used to study therapeutic (nutritional) approaches aimed at reducing ICU-Acquired Weakness.

Abstract 5 (2023 ESPEN)

Topic: Critical Care

Abstract Submission Identifier: ESPEN23-ABS-2014

Title:
Does whole body measured splanchnic extraction of amino acids with stable isotopes tracers in the prandial state represent tracee splanchnic extraction?

Authors:
Gabriella A.M. ten Have, John J. Thaden, Mariëlle P.K.J. Engelen, Nicolaas E.P. Deutz

Affiliations:

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

Rationale. Establishing the metabolic fate of nutrients coming from food intake is critical for the interpretation of the benefits of a food intervention. With a combination of enteral and parenteral administration of tracer stable isotopes like L-phenylalanine (Phe) during feeding, first pass tracer splanchnic extraction (SPE) of that amino acid can be determined on a whole body level (WbSPE). Tracer WbSPE is often suggested to relate to the tracee SPE, but conducting invasive studies across the human splanchnic area to confirm this are difficult. Therefore, we studied in the pig the relationship between tracer WbSPE and the first, second pass, and total tracer/tracee SPE. Secondly, we studied the SPE dynamics in the early recovery of *Pseudomonas* induced sepsis.
Methods. In 43 healthy or septic catheterized pigs (±25 kg), primed-continuous IV infusions of L-[ring-D_5]-Phe and enteral L-[1-^{13}C]-Phe in an amino acid mixture (per kg/hour: 0.031 gr N, 0.78 gr maltodextrine) was administered for 6 hours. At tracer steady-state, arterial and hepatic vein blood was collected. Plasma enrichments/ concentrations: LC-MS/MS, plasma flow: PAH dilution, statistics: mean [95%CI]; α=0.05. Pearson correlation, ANOVA.
Results. Tracer WbSPE was related to the first pass tracer SPE (r=0.306, p=0.046), but not to tracee SPE (r=0.191, p=0.220). Tracer WbSPE was lower than tracer total SPE (p=0.0152), but comparable to first pass SPE. In the early sepsis recovery, WbSPE was 28% [12,44] (p=0.0011) higher but not the tracer total SPE.
Conclusions. Tracer WbSPE does not reflect the tracee SPE, but only first pass SPE. Early sepsis recovery changes the dynamics between first and second pass tracer SPE. Our observations cast doubt on whether tracer WbSPE can be used to interpret the anabolic benefits of a protein meal.