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TITLE: Ghrelin Signaling Regulates Microbiome-Gut-Brain Axis in Inflammatory Bowel Disease and Post-Traumatic Stress Disorder

PRINCIPAL INVESTIGATOR: Chia Shan Wu

CONTRACTING ORGANIZATION: Texas AgriLife Research

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Chia-Shan Wu

E-Mail: ChiaShan.Wu@ag.tamu.edu

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Texas A&M AgriLife Research
Department of Nutrition
123 Cater-Mattil, 2253 TAMU
College Station, TX 77843

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14. ABSTRACT
Gut microbiota is a critical regulator of host's metabolism, immune system and cognitive function. However, the microbiome-gut-brain axis has not been systematically studied in Inflammatory Bowel Disease (**IBD**), much less in Post-traumatic stress disorder (**PTSD**). A novel experimental "2-hit" model (**IBD-PTSD**) is established, where mice are subjected to dextran sulfate sodium (**DSS**)-induced ulcerative colitis and then conditioned fear (**CF**) memory test, to study the role of microbiome-gut-brain axis in these inflammatory pathologies. We found that DSS-induced colitis led to contextual memory deficit in both male and female mice, even when colitis-associated disease symptoms such as diarrhea and rectal bleeding have subsided. Molecular characterization showed prolonged neuroinflammation and astrogliosis in the mice that had been exposed to DSS. These data have been published in the peer-reviewed journal *Molecular Brain*. New data showed that ghrelin attenuated DSS-induced colitis, exerting anti-inflammatory effects in the recovery phase, and promoting tissue repair in part through regulating epithelial metabolism via PPARgamma mediated signaling; however, ghrelin treatment is insufficient to rescue neuroinflammation induced by peripheral inflammation.

15. SUBJECT TERMS
Inflammatory Bowel Disease (IBD); Posttraumatic stress disorder (PTSD); Ulcerative colitis; Gut microbiome, Ghrelin

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Clinical and pre-clinical data pin-point inflammation as the main disease condition underlying inflammatory bowel disease (IBD) and Post-traumatic stress disorder (PTSD), which predisposes the subject to further inflammatory pathologies. The central hypothesis of the current research is that gut microbiota dysbiosis and associated intestinal inflammation is the unifying factor contributing to pro-inflammatory pathologies underlying IBD and PTSD. Hence, agents that promote rebalancing of the microbiome could ameliorate disease symptoms. A novel experimental model of IBD (dextran sulfate sodium-induced ulcerative colitis) followed by Pavlovian fear conditioning and fear recall testing (referred to as IBD-PTSD paradigm) will be established and used to test the hypothesis as well as the therapeutic potential of the gut hormone ghrelin.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Inflammatory Bowel Disease (IBD); Posttraumatic stress disorder (PTSD); Gut microbiome, Ulcerative colitis; Ghrelin.

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major goals:

- **Aim 1.** Define the dynamic and temporal changes in microbiota composition, metabolomics, inflammation and fear memory in experimental IBD-PTSD. (**Year 1**)
- **Aim 2.** Evaluate the ability of ghrelin to ameliorate experimental IBD-PTSD. (**Year 2**)

Updated Statement of Work for Year 1 (updates in the "Progress" column).

Specific Aim 1: *Define the dynamic and temporal changes in microbiota composition, metabolomics, inflammation and fear memory in experimental IBD-PTSD*	**Proposed Timeline**	**Progress**	**Site 1**
Major Task 1: *in vivo* experiment 1	Months	Calendar	
Local IRB/IACUC Approval	1-2	Approved on 01/17/2020	
ACURO Approval	2-3	Approved on 03/18/2020	
Subtask 1: Breeding of C57BL/6 mice for experimental group	3-4	Jun-Jul	Dr. Wu (n=40)
Subtask 2: Switch to open source diets and monitor body weight and body composition until mice reach 12 weeks of age.	4-6	Jul-Sep	Dr. Wu
Subtask 3: Perform animal experiment DSS-fear conditioning, sample collection	7-8	Oct-Dec	Dr. Wu
Milestone(s) Achieved: completion of sample and data collection from *in vivo* experiment 1.	8	Dec	

Major Task 2: molecular and biochemical characterization			
Subtask 1: fecal microbiome characterization	9-12	planned	Dr. Wu and genomics core facility
Subtask 2: fecal metabolome characterization	9-12	planned	Dr. Wu and metabol-omics core facility
Subtask 3: multiplex analysis of serum cytokines and gut hormones, plasma acyl-ghrelin levels	9-12	90%	Dr. Wu
Subtask 4: Molecular characterization of colons: histology, qPCR	9-12	90%	Dr. Wu
Subtask 5: Molecular characterization of brain: histology, qPCR	9-12	90%	Dr. Wu
Milestone(s) Achieved: completion of sample processing and molecular characterization work. Completion of data analysis.	12	delayed	

Updated Statement of Work for **Year 2** (updates in the "Progress" column).

Specific Aim 2: *Evaluate the ability of ghrelin to ameliorate experimental IBD-PTSD*	**Proposed Timeline**	**Progress**	**Site 1**
Major Task 3: *in vivo* experiment 2			
Subtask 1: Breeding of C57BL/6 mice for experimental group	1-2	Jan-Feb	Dr. Wu (n=50)
Subtask 2: Switch to open source diets and monitor body weight and body composition until mice reach 12 weeks of age.	2-4	Feb-Apr	undergrads
Subtask 3: Perform animal experiment DSS-fear conditioning, ghrelin injection, sample collection	5-6	May-Jun	Dr. Wu and undergrads
Milestone(s) Achieved: completion of sample and data collection from *in vivo* experiment 2.	7	completed	
Major Task 4: molecular and biochemical characterization			
Subtask 1: fecal microbiome characterization	9-12	planned	Dr. Wu and genomics core facility
Subtask 2: fecal metabolome characterization	8-9	planned	Dr. Wu and metabolomi cs core facility
Subtask 3: multiplex analysis of serum cytokines and gut hormones, plasma acyl-ghrelin levels	9-12	50% complete	Dr. Wu and undergrads
Subtask 4: Molecular characterization of colons: histology, qPCR	9-12	50% complete	Dr. Wu and undergrads
Subtask 5: Molecular characterization of brain: histology, qPCR	9-12	50% complete	Dr. Wu and undergrads
Milestone(s) Achieved: completion of sample processing and molecular characterization work. Completion of data analysis.	12	Delayed	

Updated Statement of Work for **Year 3** (No-cost extension year).			
Specific Aim 2: *Evaluate the ability of ghrelin to ameliorate experimental IBD-PTSD*	**Proposed Timeline**	**Progress**	**Site 1**
Major Task 4: molecular and biochemical characterization			
Subtask 3: multiplex analysis of serum cytokines and gut hormones, plasma acyl-ghrelin levels	12	completed	Dr. Wu and undergrads
Subtask 4: Molecular characterization of colons: histology, qPCR	12	completed	Dr. Wu and undergrads
Subtask 5: Molecular characterization of brain: histology, qPCR	12	completed	Dr. Wu and undergrads
Milestone(s) Achieved: completion of sample and data collection from *in vivo* experiment 2. completion of sample processing and molecular characterization work.	12	completed	

What was accomplished under these goals?

In the reporting period 03/01/2022-02/28/2023 (no-cost extension year), the COVID-19 pandemic has continued to impact the progress of research work; as a result, the microbiome and metabolome work with core facilities were delayed (staffing shortage, machine break-down with no replacement parts available). The PI continued to perform most of the research activities, with assistance from undergraduate students under the PI's direction.

In this project (award performance period 03/01/2020-02/28/2023), the following research activities had been carried out:

Aim1. Define the dynamic and temporal changes in microbiota composition, metabolomics, inflammation and fear memory in experimental IBD-PTSD.

Research Design: Male (M) and female (F) C57BL/6 mice were switched to and maintained on semi-purified regular diet from 4-weeks of age. At 12-weeks old, mice were randomly separated into 4 groups (M-control, F-control, M-DSS, F-DSS). Mice were treated with normal (control) or 2% dextran sodium sulfate (DSS) in drinking water for 6 days (Day 0-6) to induce colitis as shown in **Figure 1** and switched back to normal drinking water until end of study. Body weight and disease activity scores (including body weight, fecal consistency and rectal bleeding), a measure of the physical status and clinical symptoms of the animals, were evaluated daily for the entire duration of the study. Conditioned Fear (CF) memory tests were performed from day 15 to day 16 after the start of DSS treatment, when clinical symptoms had receded, and tissue repairs were occurring.

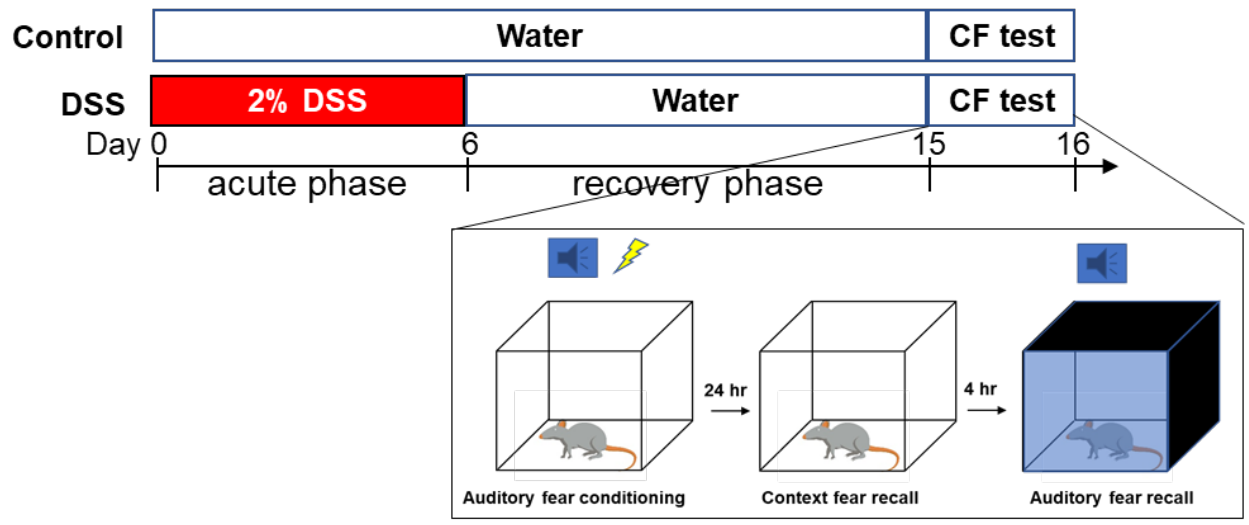
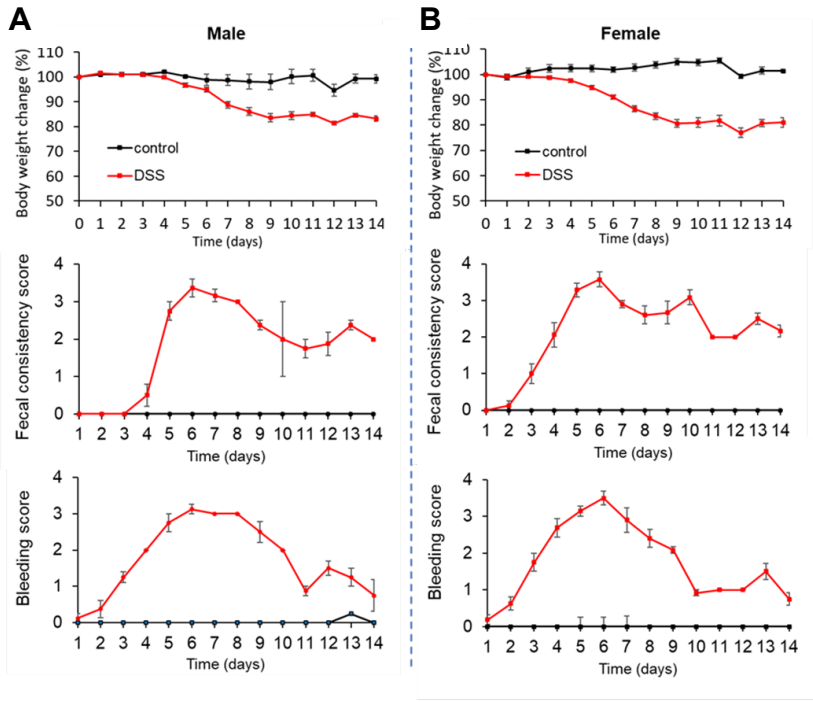


Figure 1. Schematic diagram of experimental IBD-PTSD model.
Mice were given normal (control) or 2% DSS in drinking water for 6 days (Day 0-6), then switched to normal drinking water and allowed to recover, mimicking clinical remission. On Day 15, mice were subjected conditioned fear (CF) experiments. Mice were conditioned to an auditory cue followed by mild foot-shock; 24 hours later, context and auditory fear recall tests were performed. Mice were sacrificed on Day 17.

Our data showed that following an active episode of DSS-induced colitis, both male and female mice had recovered from diarrhea (fecal consistency score < 3) and rectal bleeding (bleeding score <2) by Day 10; however, there were still mild symptoms present, including soft and darkened stool, and reduced body weight (**Figure 2**).



Fecal Score	
0	Normal Stool
1	Soft but Formed Pellet
2	Very Soft Pellet
3	Diarrhea (No Pellet)
4	Dysenteric Diarrhea (Blood in diarrhea)

Rectal Bleeding Score	
0	No Bleeding
1	
2	Presence of Visible Blood in Stool
3	
4	Gross Macroscopic Bleeding

Figure 2. Pilot IBD-PTSD experiment in C57BL/6 mice.
Disease activities, including body weight, fecal consistency and rectal pathologies were monitored everyday, data from male mice were shown in (**A**) and female mice in (**B**).

In addition, our data showed that in the remission period after an active episode of colitis, hippocampus-dependent contextual fear memory was significantly decreased in the colitis-exposed mice (**Figure 3A**), in both male and female mice. On the other hand, auditory fear memory (amygdala-dependent) were not significantly different between control and DSS-exposed mice (**Figure 3B**). These data suggest that a prior episode of gut inflammation did not impact the amygdala-dependent fear memory function, but affected hippocampus-dependent memory function in female mice.

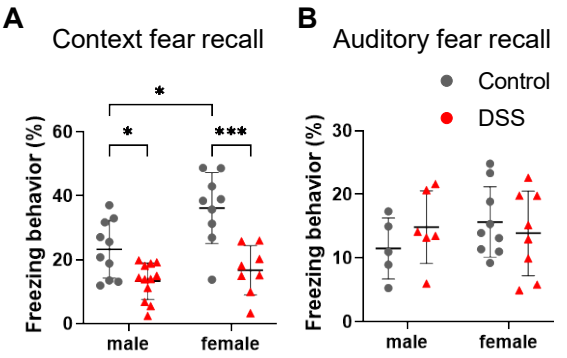


Figure 3. Prior exposure to DSS-induced colitis led to impaired hippocampus-dependent contextual memory. DSS-exposed mice showed impaired hippocampal-dependent contextual fear memory (**A**), while amygdala-dependent auditory fear memory remained intact.

Downstream analyses were carried out to assess neuroinflammation in the hippocampal regions. Our data showed that DSS-exposed mice, at Day 17, had marked signs of astrogliosis (increased GFAP-labeled cells) in the hippocampal regions compared to control (**Figure 4A**). To characterize the dynamics of the neuroinflammatory response to peripheral inflammation further, we performed additional *in vivo* experiments, varying the time of tissue collection after an active episode of colitis. Our data showed that there were time-dependent increases in the expression of inflammatory genes *Nfkb*, *Trem2* (microglial marker), *IL-1b*, and *GFAP* (astrocyte marker). Interestingly, expression of *Nfkb* and *Trem2* subsided at Day 42, while *IL-1b* and *GFAP* expression levels remained significantly elevated (**Figure 4B**).

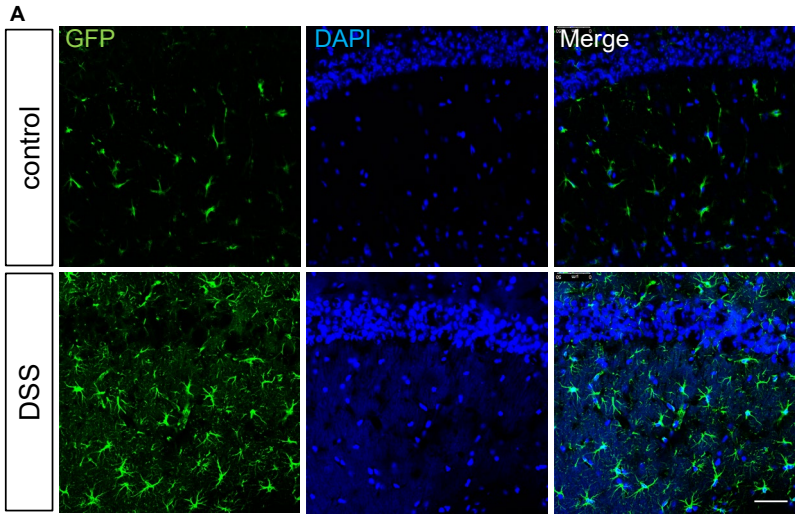


Figure 4. Prior exposure to DSS-induced colitis led to neuroinflammation in the hippocampal regions. Mice were given normal (control) or 2% DSS in drinking water for 6 days, then switched back to normal drinking water to recover. **(A)** Representative images of the hippocampal regions of mice at Day 17. DSS-exposed mice showed increased astrogliosis (increase in abundance and cell size of GFAP-labeled astrocytes (green). Sections were counterstained with DAPI (blue). Scale bar 50 µm. (**B**) Expression of inflammatory genes *Nfkb*, *Trem-2*, and *IL-1b* in the hippocampus of mice at Day 10, 17 and 42. N=4 per group, * p<0.05, control *vs.* DSS.

Summary for Aim 1:

- Our results demonstrated that DSS-induced colitis leads to prolonged neuroinflammation and impaired contextual fear memory.
- These data were published in the peer-reviewed journal *Molecular Brain*, 2022.

Aim 2. Evaluate the ability of ghrelin to ameliorate experimental IBD-PTSD.

Research Design: Male (M) and female (F) C57BL/6 mice were fed and maintained on semi-purified regular diet from 4-weeks of age. At 12-weeks old, mice will be randomly separated into 6 groups (M-control-vehicle, M-DSS-vehicle, M-DSS-ghrelin, F-control-vehicle, F-DSS-vehicle, F-DSS-ghrelin). All mice were treated with normal (control) or 2% DSS (DSS) in drinking water for 7 days (Day 0-7) to induce colitis, then switched to normal drinking water, and body weight and DAI scores evaluated daily as in Aim 1. Ghrelin (2 nmol/mouse) were daily injected intraperitoneally for 9 consecutive days, from Day 3 to Day 13 (indicated by blue arrowheads, **Figure 5A**). CF tests were performed from Day 15 to Day 16 after the start of DSS treatment.

Our data showed that acyl-ghrelin treatment, at a dose that we had previously shown to protect against muscle atrophy, attenuated DSS-induced colitis (**Figure 5B-C**). Ghrelin treatment promoted colon tissue repair from DSS-induced colitis (**Figure 5D**) and reduced inflammation (**Figure 5E-F**); however, ghrelin treatment did not rescue impaired contextual fear memory (**Fig. 5G**).

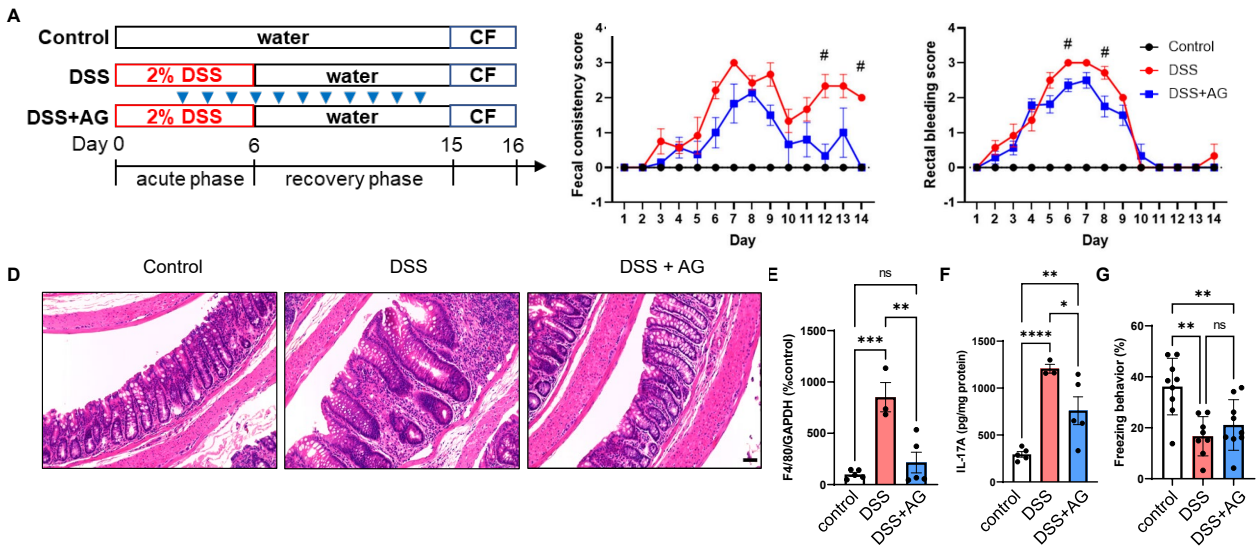


Figure 5. Ghrelin reduced DSS-induced colitis and promoted tissue repair, but did not rescue impaired contextual fear memory. (**A**) Schematic diagram of experimental IBD-PTSD model. Mice are given normal (control) or 2% DSS in drinking water for 6 days (Day 0-6), then switched to normal drinking water and allowed to recover, mimicking clinical remission. Mice in DSS + AG group were intraperitoneally injected with ghrelin daily from days 3–13, indicated by blue arrowheads. Ghrelin alleviated disease activities including fecal consistency (**B**) and rectal bleeding (**C**), promoted colon tissue repair (**D**), and decreased colon inflammation, indicated by reduced levels of F4/80 (**E**) and IL-17A (**F**). Mice were subjected to conditioned fear (**CF**) test. DSS-exposed mice showed impaired hippocampal-dependent contextual fear memory (**G**); ghrelin treatment had no effect.

Extending this finding, we also showed that ghrelin knockout (Ghrl KO) mice exhibited exacerbated disease activities including loss of body weight, fecal consistency and rectal bleeding score (**Figure 6**).

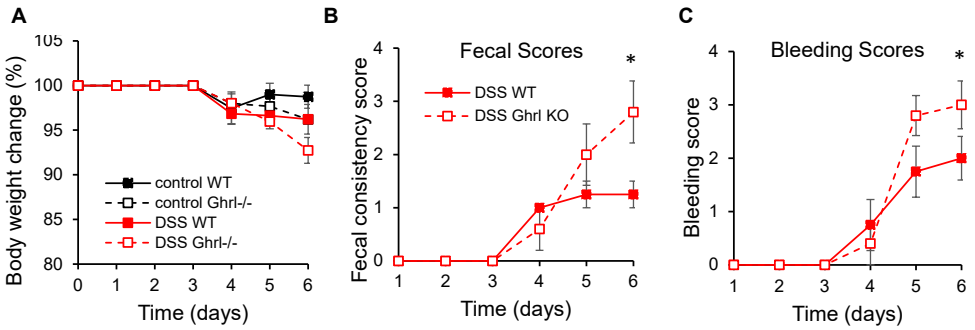


Figure 6. DSS-induced colitis in WT and Ghrl KO mice.
Normal (control) or 2% DSS in drinking water was given to mice for 6 days, and water was changed every 2 days. Disease activities were monitored everyday, including body weight (**A**), stool consistency (**B**) and rectal bleeding (**C**). N=5-6, control *vs.* DSS, $p<0.05$.

Overall, these data suggested that 1) endogenous ghrelin plays a role in intestinal homeostasis, and 2) ghrelin, at pharmacological doses that are above endogenous levels, partially protected against DSS-induced colitis.

The protective effect of ghrelin on DSS-induced colitis seemed relatively mild. Whether this is due to down-regulation of ghrelin receptor (namely the Growth Hormone Secretagogue Receptor, GHS-R) after repeated dosages of ghrelin is unknown. Interestingly, previous collaborative work with Dr. Yuxiang Sun on global ablation of GHS-R (GHS-R KO) mice showed exacerbated DSS-induced colitis as well as gut dysbiosis in the GHS-R KO mice, in line with the Ghrl KO mice data. The work on Ghrl KO was recently published in *Experimental Biology and Medicine,* 2022.

In pursuing the mechanism of how ghrelin signaling functions in maintaining intestinal homeostasis, we hypothesized that the peroxisome proliferator-activated receptor gamma (**PPARγ**) in colonic epithelial cells could be one of the target pathways mediating ghrelin's effects. PPARγ has been shown to regulate epithelial oxygenation and colonization resistance to pathogenic bacteria, and to protect against experimental IBD. We set up new *in vitro* assays in the lab and showed that ghrelin increased oxygen consumption rate in the human colon adenocarcinoma Caco-2 cells (**Figure 7**) and activated transcriptional activity of PPARγ (**Figure 8**). These data suggest that PPARγ is indeed a downstream target of ghrelin, and ghrelin could activate intestinal epithelial PPARγ to maintain healthy colonocyte bioenergetics, consuming oxygen and thereby maintain a low oxygen tension. This in turn creates a favorable intestinal microenvironment for inhabitation and adaptation of the obligate microbes. These data are now published in peer-reviewed journal *International Journal of Molecular Sciences*, Dec 2022.
We are planning further experiments to pursue this new hypothesis.

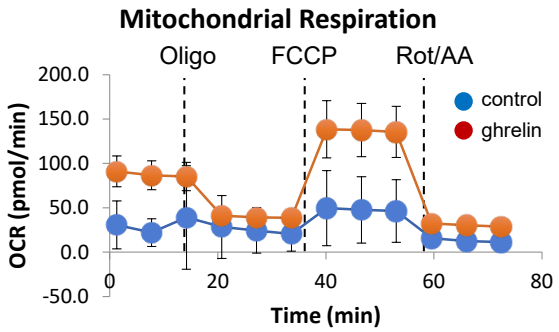


Figure 7. Cellular bioenergetics.
Caco-2 cells were treated with vehicle or 100 nM human acyl-ghrelin for 24 hours. ATP synthase inhibitor oligomycin (Oligo), mitochondrial uncoupling reagent FCCP, and ETC inhibitors rotenone/actinomycin A (Rot/AA) were injected at indicated times. Seahorse extracellular flux analysis showed that ghrelin increased oxygen consumption rate (OCR), suggesting increased mitochondrial respiration. Error bars represent SD, experiment done in 12 replicates.

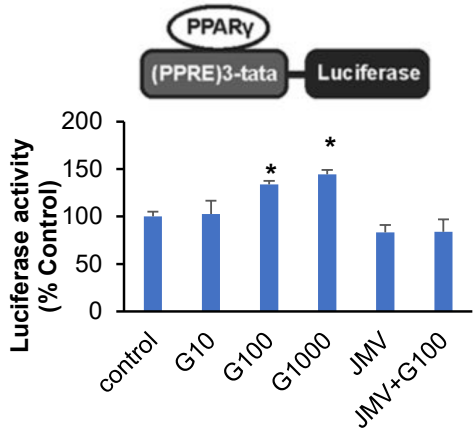


Figure 8. Ghrelin dose-dependently activated PPARγ transcription. PPARγ-reporter assay showing activation of PPARγ by ghrelin in human colon adenocarcinoma Caco-2 cells. PPRE-luciferase plasmids were transiently transfected into Caco-2 cells for 48 hours, cells were then treated for 2 hours with 10, 100 or 1000 nM of ghrelin. JMV: 3 uM of GHS-R antagonist JMV 2959. Error bars represent SEM, experiment done in triplicates, repeated 2 times. * $p<0.05$ vs. control group.

New evidence from metabolome analysis of short chain fatty acids levels in feces from mice on normal chow diet or purified control diet used in the *in vivo* studies in Aims 1 and 2 revealed that short chain fatty acids levels were significantly reduced in mice fed purified control diet. In particular, **butyric acid**, a key microbial metabolite that serves as fuels for colonic cell metabolism as well as anti-inflammatory agent in the brain, was significantly reduced in mice fed purified diets compared to those fed normal chow diet. The regular rodent chow diet typically contains 15%–25% fiber that comes from diverse sources such as ground wheat, ground corn, dehulled soybean meal, and wheat germ, while purified control diet contains 50 mg non-fermentable cellulose as fiber source. Hence, we predict that ghrelin's full therapeutic effect in rebalancing microbiota-gut-brain axis may be dependent on the presence dietary fiber to promote growth of obligate microbes and production of beneficial microbial metabolites. This remains to be further tested.

Summary: While it is known that epithelial oxygen levels are increased in colitis, precisely how the colonocyte metabolism is altered in disease remission and re-colonization of obligate (healthy) microbes remain largely undetermined. Ghrelin could activate intestinal epithelial PPARγ to maintain healthy colonocyte bioenergetics and low oxygen tension, thereby facilitates intestinal homeostasis for inhabitation and adaptation of the obligate microbes. We also envision that the new *in vitro* assays could be used in screening for compounds that target colonocyte metabolism, to improve host-microbe interactions in IBD.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The project had provided opportunities for undergraduate students to be trained in research activities. The PI provided one-on-one trainings on experimental work as well as manuscript preparation, developing their skills in literature search, critical thinking, and problem-solving.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to Report.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The preclinical data generated from this project demonstrated that peripheral inflammation, in the form of DSS-induced ulcerative colitis, could induce prolonged neuroinflammation, negatively impacting hippocampal memory function. These data add to the existing knowledge on the gut-brain axis, and suggest that in addition to clinical management of the symptoms of IBD, other strategies to monitor and reduce neuroinflammation may need to be considered to prevent potential progression to chronic disease conditions such as dementia or neurodegenerative diseases.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Due to the COVID-19 pandemic, research work has been disrupted and delayed. These delays have significantly impacted on the timeline of the animal experiments and subsequent downstream analyses.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Due to the COVID-19 pandemic, research work has been disrupted and delayed. These delays have significantly impacted on the timeline of the animal experiments and subsequent downstream analyses, as well as hiring staff.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

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.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Muthyala, S. D.V., Shankar, S., Klemashevich, C., Blazier, J. C., Hillhouse, A., **Wu, C.-S.** Differential Effects of the Soluble Fiber Inulin in Reducing Adiposity and Altering Gut Microbiome in Aging Mice. Journal of Nutritional Biochemistry 105: 108999, 2022, published (https://doi.org/10.1016/j.jnutbio.2022.108999); federal support acknowledged.

2. Tuchaai, E., Endres, V., Jones, B., Shankar, S., Klemashevich, C., Sun, Y., **Wu, C.-S.** Deletion of ghrelin alters tryptophan metabolism and exacerbates experimental ulcerative colitis in aged mice. Experimental Biology and Medicine, 247 (17), 1558-1569, 2022, published; federal support acknowledged.

3. **Wu, C.-S.#**, Endres, V. Prior episode of colitis impairs contextual fear memory. Molecular Brain 15:74, 2022. # Corresponding author; published; federal support acknowledged.

4. Muthyala, S., Chapkin, R.S., Wu, C., **Wu, C.-S.** Ghrelin alleviates experimental ulcerative colitis in old mice and modulates colonocyte metabolism via PPARγ pathway. International Journal of Molecular Sciences 24 (1): 565, 2023, published; federal support acknowledged.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic*

information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention,

diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	*Chia Shan Wu*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*ORCID ID: 0000-0002-6034-939X*
Nearest person month worked:	*16*
Contribution to Project:	*Dr. Wu has performed all work described in the award period.*
Funding Support:	*Dr. Wu is also supported by AG061726A (R21), National Institute on Aging.*

Name:	*Valerie Endres*
Project Role:	*Undergraduate student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Valerie has performed some of the research activities described in the award period.*
Funding Support:	*Not applicable.*
Name:	*Brock Jones*
Project Role:	*Undergraduate student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Brock has performed some of the research activities described in the award period.*
Funding Support:	*Not applicable.*
Name:	*Srilakshmi Muthyala*
Project Role:	*Undergraduate student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Srilakshmi has performed some of the research activities described in the award period.*
Funding Support:	*Not applicable.*
Name:	*Grant McCrea*
Project Role:	*Undergraduate student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Grant has performed some of the research activities described in the award period.*
Funding Support:	*Not applicable.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Award chart, Quad chart and journal article published in the award period are attached to the report.