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PRINCIPAL INVESTIGATOR: Holly Colognato

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This research study explores ways to enhance myelin repair. The premise upon which the research is founded is that the signaling molecule, Csk, acts as an endogenous suppressor of myelin repair. We have been exploring the possibility that the AMPK pathway is key component of the Csk-induced brake to myelin repair. We have activated AMPK signaling following myelin damage and preliminary indications are that AMPK activation promotes myelin health and/or repair. We also found that AMPK activation enhances oligodendrocyte differentiation from oligodendrocyte progenitor cells using rodent cultures. We have further found that AMPK activation in mice aids in myelin repair. Lastly, we have found that AMPK activation promotes metabolic alterations in developing oligodendrocytes, and likely represents, at least in part, an important mechanism to drive enhanced myelin repair. This last hypothesis will form the basis for our next study.

15. SUBJECT TERMS
Remyelination, AMPK, cuprizone

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	19	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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

The research study explores ways to enhance myelin repair by modulating cellular signaling. The premise is that the kinase Csk acts as an endogenous suppressor of myelin repair. The purpose of the study was therefore to examinet the molecular pathways that were altered by Csk loss in oligodendrocytes, the myelinating cells of the central nervous system, and to explore the possibility that the AMPK signaling pathway is a key component of the Csk-induced brake on myelin repair. We plan to determine whether AMPK signaling promotes myelin repair following cuprizone-mediated demyelination using both loss- and gain-of-function approaches. We also plan to identify

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

Myelin repair, signal transduction, AMPK, Csk, oligodendrocyte, cuprizone, metabolism, glycolytic

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.

The major goals of the project were divided into the two following specific aims, which were each further subdivided into two major tasks, as follows:

- Specific Aim 1: Determine whether AMPK signaling promotes myelin repair
  - Major Task 1: Determine the effect of AMPK loss-of-function on oligodendrocyte dynamics and myelin repair following cuprizone-mediated demyelination (~20% complete)
  - Major Task 2: Determine the effect of AMPK activation on oligodendrocyte dynamics and myelin repair following cuprizone-mediated demyelination (~90% complete)
- Specific Aim 2: Uncover novel molecular effectors that contribute to the ability of Csk-KO oligodendrocytes to undergo enhanced myelin repair.
  - Major Task 3: Perform screen to detect molecular changes in acutely isolated Csk-KO oligodendrocytes from brains undergoing myelin repair. (~20% complete)
  - Major Task 4: Perform screen to detect molecular changes in cultured oligodendrocytes following acute knockdown of Csk using siRNA. (~30% complete)

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

During the first several months of the grant period (July 1 – Oct 15, 2018), we were in the process of obtaining regulatory approval for mouse work, which essentially comprises all the experiments in the project, so we could not begin the experiments. However, during this time we were still able to test out several key antibody reagents on other tissue samples that we had in the lab, as well as research and optimize experimental strategies, all in preparation for the approved work.

Once regulatory approval was obtained, we began to do project experiments. In the remainder of year 1 (late 2018 thru June 2019) we focused on Major Task 2, which is to determine the effect of AMPK activation on oligodendrocyte dynamics and myelin repair following cuprizone-mediated demyelination. We felt these experiments "gain-of-function" experiments were the ones most likely to lead to beneficial

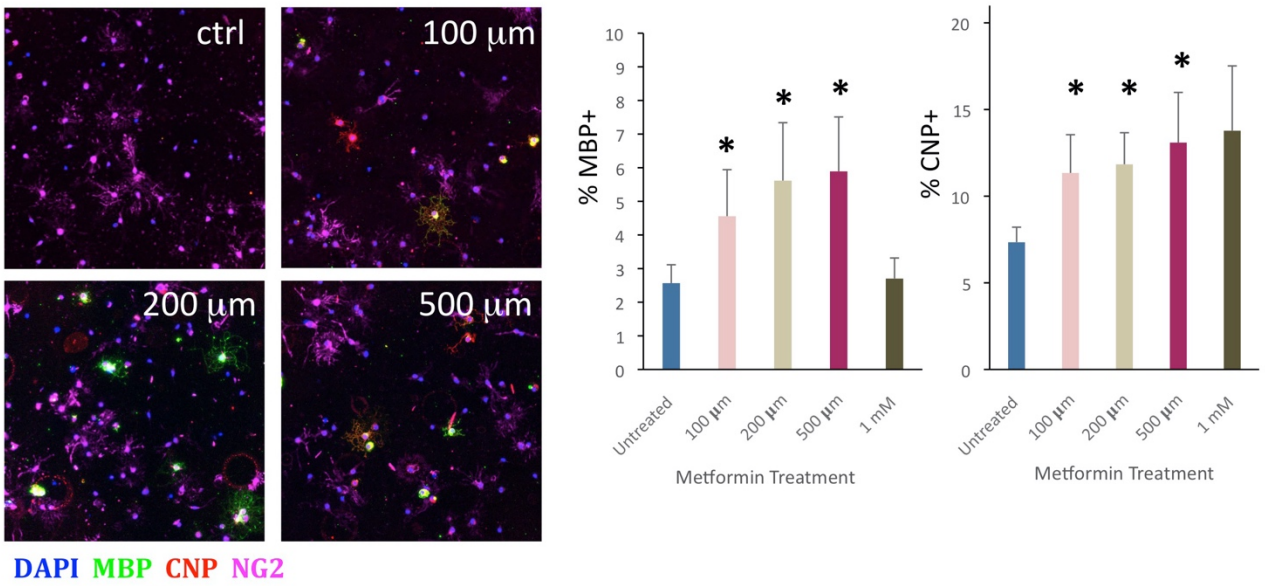

Figure 1. Dose-dependent enhancement of oligodendrocyte differentiation in response to metformin

outcomes for future translational MS research, since our preliminary data suggested that AMPK activation could jumpstart oligodendrocyte differentiation, which would have a positive outcome if applied to a demyelinating conditions.

First, we performed several rounds of small pilots to assess dosing and timing the AMPK activator, metformin, when delivered in vivo to adult mice via daily drinking water. We next assessed remyelination in the corpus callosum following cuprizone-mediated demyelination, in the presence or absence of metformin. Excitingly, our examination of myelin content of the corpus callosum indicates that metformin treatment enhances myelin levels. We now plan to follow up this observation by performing more rigorous myelin analysis such as TEM. Given the timing of metformin administration (either 2- or 4-weeks into the cuprizone treatment) it is likely that the increase in myelin is due to an increase in myelin repair engaged in by newborn oligodendrocytes. Here, we have begun to assess cellular phenotypes in these tissues to make that determination, as well as plan to engage in further time points to address whether an optimal time window for metformin intervention exists. Despite these uncertainties, our experiments strongly suggest that metformin improves either the speed or degree of myelin repair. This provides the ground work for

additional studies which will be needed to understand the degree of effect as well as the cellular mechanism by which metformin may be influencing post-cuprizone myelin levels.

We have also made headway on the question of whether metformin can directly influence oligodendrocytes, and if so, at what stage and by what mechanism does this influence on oligodendrocyte dynamics occur. Initially we had assessed metformin effects using single hit dosing strategies of OPCs, followed by assessment of lineage stage gene expression (for example, see **Figure 1**, as well as additional Figures in the 2019 Annual Progress Report). Here we learned that over the short term, metformin can stimulate increases in OPC differentiation, at the level of changes in mRNA levels of myelin-related genes as well as in the percentages of cells that transition to being CNP- and MBP-positive. We normalized these mRNA data sets relative to Olig2 mRNA levels (used as a readout for oligodendrocyte lineage cells), to guard against the possibility that metformin could shift the percentages of contaminating cell types relative to the oligodendrocyte lineage population (although we did not find substantial differences in normalizing to Olig2 versus normalizing to a housekeeping gene, GAPDH). We see that metformin treatment results in increased levels of MBP and CNP mRNA at 24 hours however NG2 and PDGFRa mRNA levels do not significantly change (or slightly trend downwards). We have noted that at day 2 and beyond these changes in gene expression are less pronounced and eventually are no longer statistically significant. We reasoned that the loss of effect over time could be due to metformin stability long term at 37 degrees Celsius.

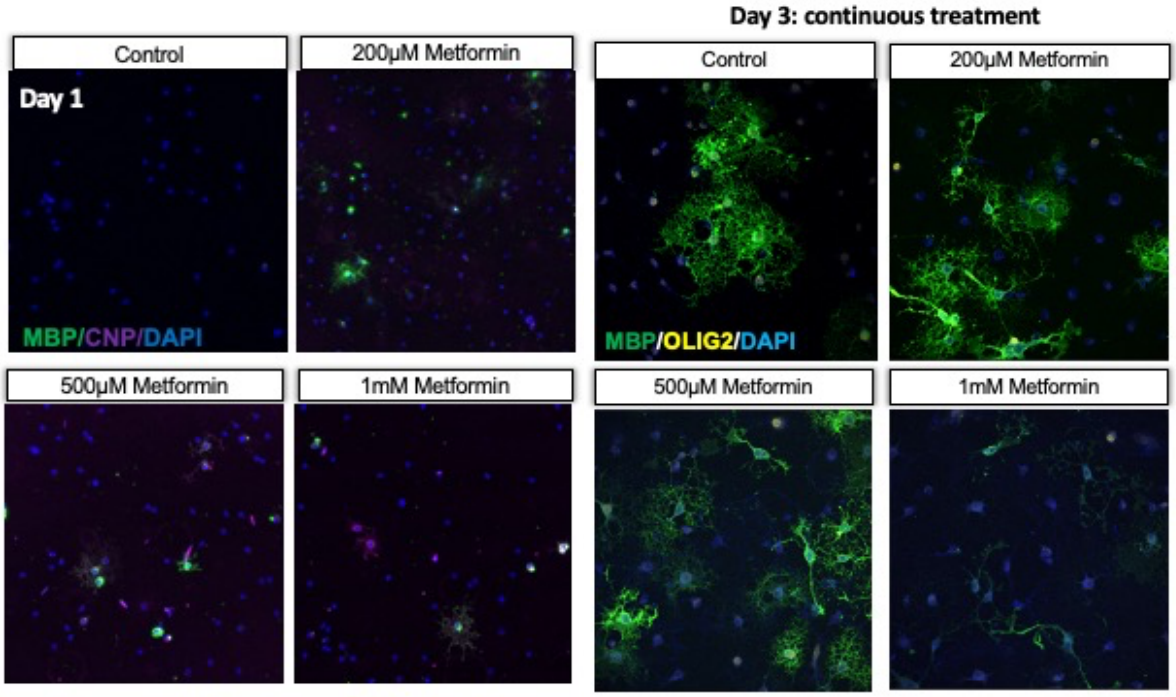

Figure 2. Oligodendrocyte differentiation in response to metformin. LEFT PANELS, OLs after 1 day of metformin treatment at indicated doses. Myelin basic protein (MBP, green), 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNP, magenta) immunoreactivity in conjunction with DAPI (blue). RIGHT PANELS, OLs after 3 days of daily metformin treatments at indicated doses. In contrast to day 1, 1 mM metformin has attenuated OL differentiation.

To counter the "diminishing returns" of metformin using an acute treatment paradigm, we switched to performing experiments in which we changed half the media, adding fresh media with and

without metformin, on a daily basis. By providing a daily dosing strategy, we found that metformin effects on lineage-specific gene expression were more robust. In addition to mRNA levels we have continued our work on assessing whether OPCs differentiate to different lineage stages (i.e., CNP-positive, MBP-positive) in response to metformin. Here we can assess individual cells rather than a cumulative level of mRNA in the whole culture, and thus this assay is predicted to be more sensitive. We find that within 24 hours in SATO+T3 (differentiation medium conditions), metformin treatment results in an increase in the percentage of cells that are either CNP-positive or MBP-positive (**Figure 2,3**). At the same time, metformin treatment results in a trend towards fewer NG2-positive cells (**Figure 3**). We are currently assessing longer term treatment of metformin using our daily dosing strategy described above.

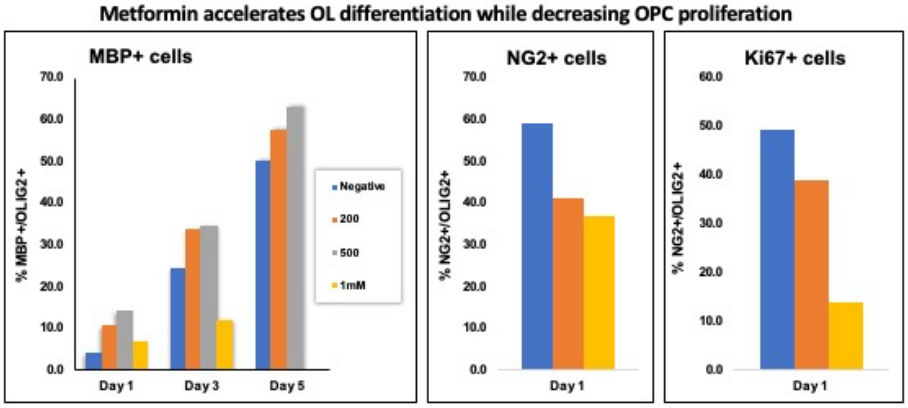

Figure 3. Oligodendrocyte lineage phenotypes in response to daily dosing of metformin. The percentage of cells that express myelin basic protein (MBP) increases with increasing time in differentiation medium (day 1 – day 5) as well as in response to 200 or 500 μM metformin. In contrast 1 mM metformin has a more limited pro-differentiation effect at day 1 but by day 3 attenuates differentiation. At day 5 many of the cells grown in daily doses of 1 mM metformin had died so analysis was not performed on the remaining cells. At day, treatment with either 200 mM or 1 mM metformin decreases the number of OL lineage cells that are NG2+ as well as substantially decreases proliferation, as indicated by Ki67 immunoreactivity.

Another avenue that we have been investigating is whether metformin alters the proportion of oligodendrocytes by affecting the small proportion of non-oligodendrocyte cells (astrocytes and microglia) in our primary cultures. In other words, we wanted to determine if changes in oligodendrocyte numbers, or gene expression profiles, could simply be due to gain or loss of other cell types in the culture. With an n of 3, we do not see significant changes in either astrocytes (determined as the % GFAP+ cells) or microglia (determined as the % Iba1+ cells) in our cultures. We have also begun to assess microglial phenotypes, to see if metformin has influences on pro- or anti-inflammatory phenotypes. Thus we have determined that there is no change in the percentage of Iba1+ cells that co-express Arg1 (virtually 100%), an anti-inflammatory state readout. However the oligodendrocyte culture conditions contain

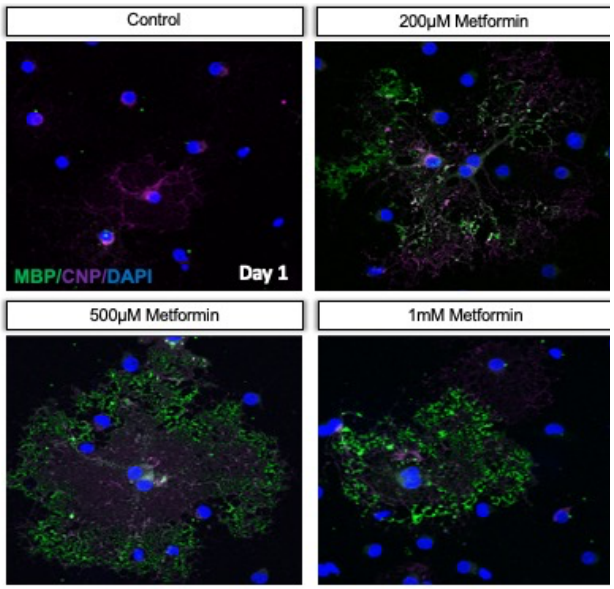

Figure 4. Oligodendrocyte morphology in response to metformin. Myelin basic protein (MBP, green), 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNP, magenta) immunoreactivity in conjunction with DAPI (blue).

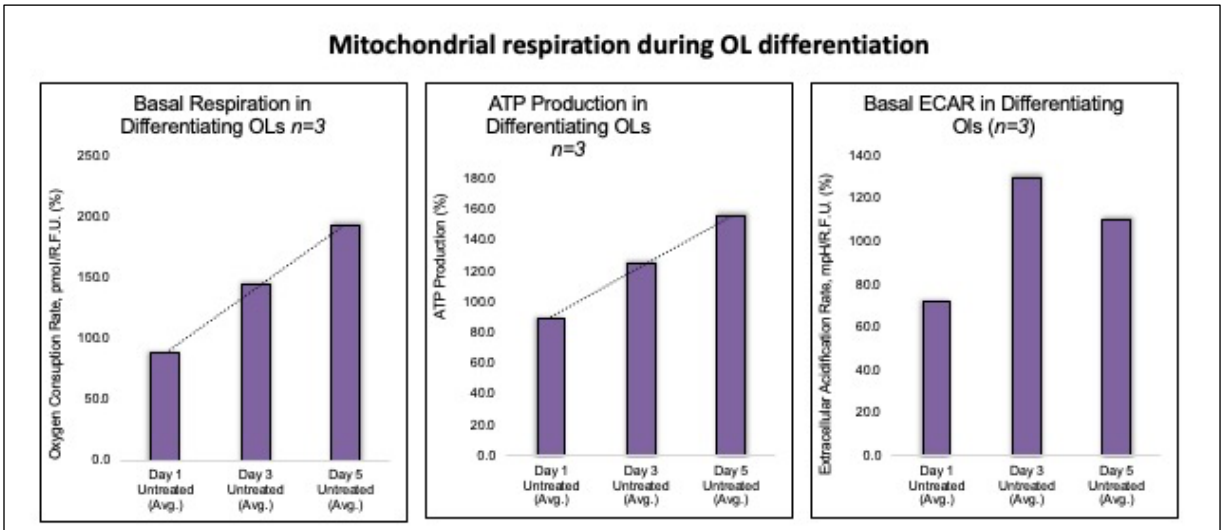

Figure 5. Changes in mitochondrial respiration as oligodendrocyte progenitor cells differentiate into oligodendrocytes (OLs). We have been using the Seahorse XF Analyzer System to measure oxygen consumption rate (OCR), ATP production, and extracellular acidification rate (ECAR) of live oligodendrocytes, seeded at 20,000 cells per well in a multi-well plate. We find that all 3 measures increase during oligodendrocyte differentiation, with basal respiration and ATP production increasing gradually whereas basal ECAR increases sharply between day 1 and day 3 differentiation then levels off as cells mature.

progesterone, which is a strong driver of the Arg1+ anti-inflammatory microglial state, and may not be the best scenario to assess potential effects of metformin on microglia. We will therefore continue to assess the “big picture” of all relevant cells, including microglia, in as we assess our mice that have undergone cuprizone treatment in the presence or absence of metformin.

In addition to changes in protein expression we have also noted that metformin leads to substantial changes in oligodendrocyte morphology (**Figure 4**). We have therefore performed several experiments to explore morphometric analysis of oligodendrocytes treated with metformin, and by Sholl analysis we have found that oligodendrocyte branching is increased in oligodendrocytes treated with metformin (not shown). We are currently gearing up to assess oligodendrocyte morphology in vivo in response to metformin, also using Sholl analysis, but here we plan to use Neurolucida software to better assess branching in vivo.

An exciting development that has arisen from these studies is the consideration of how metformin may be promoting oligodendrocyte differentiation through inducing metabolic flexibility. AMPK acts as the metabolic sensor in the cell and is activated during energy intensive processes (e.g., myelination) to regulate the activity or expression of proteins involved in ATP synthesis. Once activated, AMPK inhibits anabolic processes and bias the cell towards

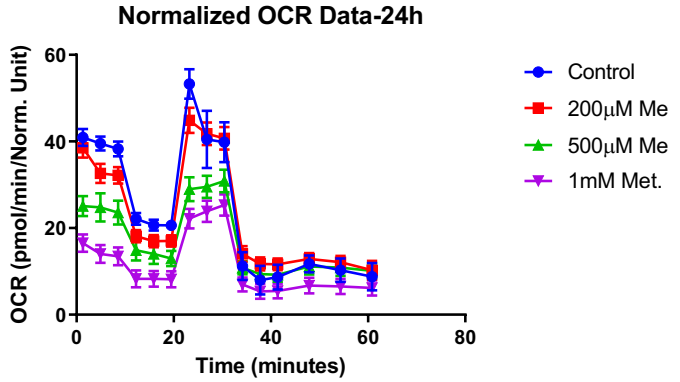

Figure 6. Seahorse SF Analyzer provides mitochondrial stress test profiles of oligodendrocytes following metformin treatments. *OCR*, oxygen consumption rate.

catabolic reactions for the generation of ATP. We hypothesized that exogenous activation of oligodendrocyte AMPK using metformin, will induce metabolic changes, and may do so in a way that benefits the cell during high metabolic demand processes such as differentiation. We have therefore used our same oligodendrocyte preparations, treated with different doses of metformin for different time points, to determine the effect of AMPK stimulation on oligodendrocyte metabolism. We have also been characterizing the changes in oligodendrocyte metabolism that occur during differentiation without treatment (**Figure 5**). Briefly, OLs are subjected to a mitochondria stress test by transient exposure to a 2μM dose of oligomycin to inhibit ATP synthase of the electron transport chain (ETC). This allows one to measure differences in oxygen consumption rates compared to basal respiration to calculate ATP production. For measuring changes in spare respiratory capacity, we inject the uncoupling agent FCCP at a concentration of 2μM. Finally, to adequately assess metabolic differences in these cells, we use a combination of the drugs rotenone and antimycin A (Rot/AA) at 0.5μM to calculate nonmitochondrial respiration driven by other cellular processes. Plating conditions and drug concentrations have been established by our initial optimization experiments.

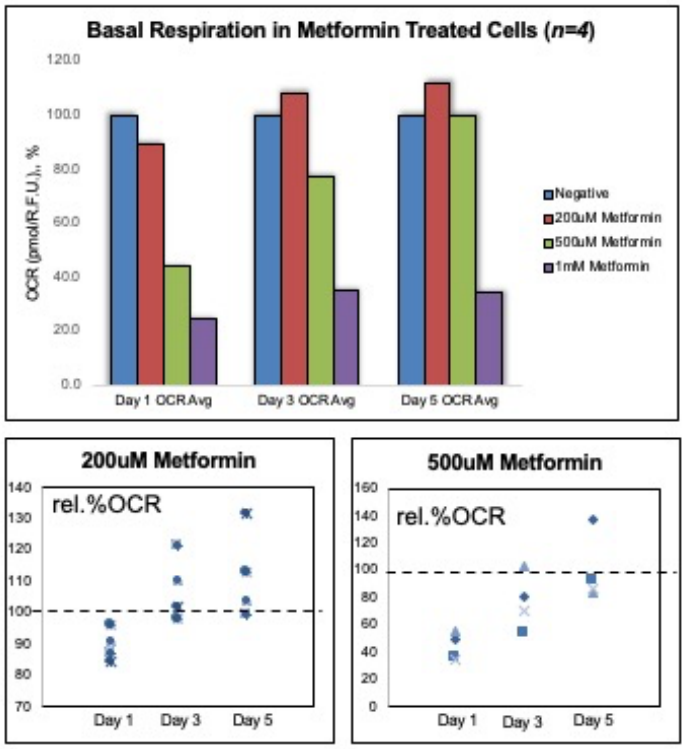

Figure 7. Mitochondrial respiration in response to metformin. Basal respiration as measured by oxygen consumption rate (OCR) on the Seahorse XF analyzer.

We next examined mitochondrial respiration in response to different doses of metformin as well as at different stages of OL differentiation. Again, we are using the Seahorse XF Analyzer to assess OL metabolism in live cells. We observe that metformin treatment at day 1 of differentiation resulted in a decreased oxygen consumption rate (OCR; see **Figures 6, 7**), which was particularly dramatic at 500 mM and 1 mM metformin. By day 3 of differentiation 200 mM now resulted in increased OCR, while higher doses continued to suppress OCR. By day 5 differentiation only 1 mM metformin suppressed OCR, and lower doses either had no effect or produced an increase. These data indicate that metformin's effect on mitochondrial respiration are complex, being both dose-dependent and OL lineage stage-dependent.

In addition to mitochondrial respiration we also measured the extracellular acidification rate, or ECAR **(Figure 8)**. ECAR was increased by metformin, at all time points and at all doses, although the effect was most pronounced using the highest dose. ECAR is a readout for glycolysis. Thus it appears that overall metformin can shift the metabolic profile of OLs, favoring increased glycolysis and decreased mitochondrial respiration. Typically, oligodendrocyte progenitor cells become more glycolytic as they differentiate into mature OLs. This metabolic shift in energy production is thought to favor lipid production at the expense of energy "efficiency", due to the inefficiency in ATP production in glycolysis compared to oxidative phosphorylation. In fact mature OLs can lose mitochondrial function altogether and still survive, and continue to metabolically support axons via

lactate shuttling. There is much evidence that OL metabolism becomes dysfunctional during MS, likely due to the inflammatory environment, which is accompanied by enhanced ROS and DNA damage. The ability of metformin to shift away from mitochondrial energy production has the potential to limit ROS and further increase lactate production. Achieving a better understanding the connection between SFK signaling, AMPK activation, and these metabolic shifts are being actively pursued.

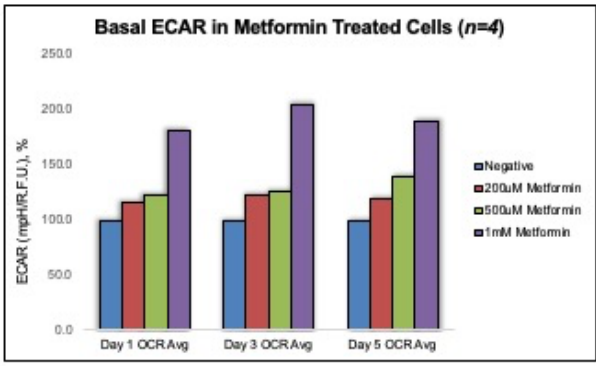

Figure 8. Basal Extracellular Acidification Rate (ECAR) is altered in metformin treated oligodendrocytes.

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.

There have been extensive training opportunities during the project. The PI has been training a graduate student in oligodendrocyte purifications and cell culture assay techniques, as well as in mouse handling, perfusions, brain tissue dissections, and processing for immunohistochemistry, as part of this project. As a result this graduate student (Mr. Narine) has been making substantial contributions to both Aim 1 and Aim 2 during the past 18 months. Mr. Narine presented his early findings as a poster presentation at the XIV European Meeting on Glial Cells in Health in Disease in July of 2019 (see conference abstract in Appendix, "An Investigation into the SFK-AMPK Signaling Axis and its Role in CNS Myelination"). Later he gave a talk on findings at the Med-into-Grad symposium at the University of Pennsylvania, November 2019 ("Exploring New Ways to Stimulate Myelin Repair in a Model of Multiple Sclerosis), as well as at in a poster presentation at the NYC Regional Glia Symposium, September 13, 2019 ("An Investigation into the SFK-AMPK Signaling Axis and its Role in CNS Myelination"). These were all highly beneficial professional development opportunities for this trainee, enabling him to discuss his work and network with top scientists in the field of Multiple Sclerosis.

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.

As mentioned in 3c, Mr. Narine, a PhD graduate student working on the project, presented his early findings as part of a poster presentation at the XIV European Meeting on Glial Cells in Health in Disease in July of 2019 ("An Investigation into the SFK-AMPK Signaling Axis and its Role in CNS Myelination"). Later he gave a talk on findings at the Med-into-Grad symposium at the University of Pennsylvania, November 2019 ("Exploring New Ways to Stimulate Myelin Repair in a Model of Multiple Sclerosis), as well as at in a poster presentation at the NYC Regional Glia Symposium, September 13, 2019 ("An Investigation into the SFK-AMPK Signaling Axis and its Role in CNS Myelination"). In addition, the Project PI discussed the project's findings at the International Society for Neurochemistry Myelin satellite meeting, Montreal in August 2019, ("Outside looking in: exploring external inputs on myelin"). In addition, the PI discussed findings with a non-expert group of undergraduates at Stony Brook University in Spring 2020 as part of the Undergraduate Pharmacology major "Colloquium in Pharmacology Research" course.

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).

There is considerable interest in the Multiple Sclerosis research community in finding mechanisms that may promote oligodendrocyte health, maturation, and capacity to ensheath multiple neuronal axons with myelin (i.e., myelination capacity). Therefore our findings that metformin, which activates the AMPK "energy sensor" pathway in cells, enhances oligodendrocyte maturation has the potential to open further inquiry into the role of energy utilization and production in key oligodendrocyte behaviors including myelination.

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.

Given the potential effect of metformin on oligodendrocyte development we think that metformin will be useful in other disease conditions in which oligodendrocyte pathology or dysfunction plays a role, such as Alzheimer's disease.

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.

As previously discussed in the Annual Report (summer 2019) we added a panel of metabolic analysis to our study of oligodendrocytes that have alterations in AMPK activity. Given the central role of AMPK in cellular energetics we took the opportunity of interacting with a neighboring lab who uses the Seahorse assay to assess changes in energy production, consumption, mitochondrial respiration, and other parameters of cellular energetics such as the degree to which glycolysis is utilized for energy production. After some initial pilot experiments, we then added the Seahorse to our "oligodendrocyte culture assay workflow". Because the Seahorse assays use a small well format with very few cells, we were able to easily add this in to our cellular analysis plans using the same number of rodent preps of primary oligodendrocytes. This addition, with the same cellular materials, gave us further insight into how AMPK changes may regulate oligodendrocyte health and metabolic abilities. This additional analysis revealed some striking changes in metabolic flexibility in response to AMPK activation, which is predicted to have a profound impact on remyelination capacity.

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

We had delays in obtaining the AMPK conditional knockout line to be used in Aim 1A, which ultimately caused us to focus more on knockdown approaches to assess AMPK loss-of-function, as well as to consider using gene editing approaches to modify AMPK subunit gene expression. In addition, due to some early encouraging findings from experiments in Aim 1B, which uses an existing mouse strain in combination with the pharmacological activation of AMPK pathways (i.e., gain-of-function), we were able to dive more deeply into the question of AMPK activation and develop a clearer picture of not only the benefits of AMPK activation to myelin repair process, but also learn some of the underlying cellular mechanisms that contribute to this benefit.

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 delay in being able to use the grant funds while awaiting ACURO approval, we were not able to use funds to support the Research Assistant working on the project in year 1. Instead he was appointed on a T32 Training Grant. Once ACURO was approved we were unable to immediately switch his support onto this award, since the T32 appointment had to go for one year. In year 2 the research assistant was moved to this award. Effort did not change (i.e., he was working on the project the whole time).

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.

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.

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).*

None to report.

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).*

None 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.*

Conference abstract from the European Meeting on Glia in Health in Disease (see appendix).

- **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.

None to report.

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

None 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.

None 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.*

None 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.)*

(1) Holly Cologanto
PI
24 months
Dr. Colognato has supervised the overall direction of the project, provided training to other project personnel, and performed experiments and analysis for the project Aims.
Funding for the PIs salary also comes from her NY state salary line as well as from NIH grants.

(2) Mohanlall Narine
Graduate Student (Research Assistant)
18 months
Mr. Narine performed experiments and analysis for the project Aims.
Funding for Mr. Narine's salary also came from an NIH Training Grant (T32) and the National Multiple Sclerosis Society.

(3) Erika Deppenschmidt
Graduate Student (Research Assistant)
4 months
Ms. Deppenschmidt performed experiments and analysis for the project Aims.
Funding for Ms. Deppenschmidt's salary also came from an NIH Training Grant (T32).

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.

Other support changes since the 2019 Annual Report:

New awards:

2020-2022	NIH, 1R21NS114769-01 (PI) "Abnormalities in postnatal brain development as a feature of congenital muscular dystrophies"
2020-2022	Department of Defense CDMRP, W81XWH2010741 (PI) "Extracellular vesicles as potential drivers of myelin health and myelin repair in pregnant MS patients"

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://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) 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.