Study Protocol

Official Title: A Pilot Study: Metformin as an immune and inflammatory modulating therapy in older adults without diabetes.

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Scientific Background

Metformin is considered first line therapy for patients with DM2 with hyperglycemia that cannot be controlled with life style alone. Unlike other oral medications, metformin is favored for its insulin sensitizing effects resulting in improved glycemic control, weight loss, and overall improvement of metabolic syndrome. Over the past fifteen years, metformin has received significant attention for its other potential therapeutic uses. Metformin has been found to decrease the rate of age related illness progression improving longevity, especially in the setting of cancer. Recent clinical trials across multiple disease states have shown metformin to decrease all-cause mortality in diabetic and non-diabetic patients.1–3 Additionally, in both animal models and human trails, metformin has been shown to decrease the risk of arterial and venous thrombosis without affecting bleeding time through its interaction with platelet mitochondria.4–6 Furthermore, treatment of diabetics with metformin has been shown to improve cognition.7 Although the mechanisms by which metformin effects longevity is an active area of both basic science and clinical research, it clearly has anti-inflammatory properties which are both independent and dependent of glycemic control.8 Recently, surgical outcomes have focused on optimizing older, deconditioned patients prior to the operation with varying protocols referred to as prehabilitation. These programs work to improve the body's response to the surgical stress resulting in improved wound healing, decreased postoperative complications, and decreased hospital length of stay. The affect of metformin, like increasing physical activity, has wide spread affects on physiology. We therefore hypothesize that metformin administration to non-diabetic adults will improve clinical outcomes to physiologic stress (including surgical stress) by improving underlying immune and inflammatory responses, that can be deleterious.

The body's response to stress is a complex interplay between the magnitude of the stress and host factors including genetics, epigenetics, age, and lifestyle. Surgical procedures result in a loss of physical barriers to the environment, local tissue injury, vascular damage, altered neurohormonal signaling, and hemodynamic changes. 9–13 Tissue injury results in stimulation of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). The systemic level of lipopolysaccharide (LPS), a ligand for TLR4, is increased following major abdominal surgery.12–14 LPS stimulation of TLR4 leads to release many pro- and anti-inflammatory cytokines, including TNF-a, IL-1, IL-6, IL-8, and G-CSF, allowing for an appropriate immune response to protect the body from invading pathogens, aid in tissue healing, and allow for overall survival. 2,3,13 However, the systemic complications of an overwhelming inflammatory response, including but not limited to acute respiratory distress syndrome, acute kidney injury, vascular thrombosis, poor wound healing both due to and independent of glycemic control, deteriorations in memory and mood, and depressed cardiac function are all well-established postoperative complications. The balance of the pro- and anti-inflammatory reaction is key to homeostasis and recovery. However, why some patients suffer from postoperative inflammatory complications while others do not is not well understood. Further, currently there is no established anti-inflammatory treatment in the peri-operative period to help alleviate or prevent an overwhelming pro-inflammatory systemic response. This study will help us to better understand the underlying mechanisms of metformin and potentially identify it as a source of pharmacologic prehabilitaiton improving surgical outcomes.

The underlying mechanisms of action of metformin are under active investigation. Presumably,

cellular mitochondria are thought to be the primary site of metformin activity. Metformin decreases intracellular AMP and increases 5'-AMP-activated protein kinase (AMPK) resulting in three main down-stream effects.15 First and foremost, there is a decrease in gluconeognesis, decreasing serum glucose levels, and improving insulin resistance. Secondly, mitochondrial AMPK activation inhibits mechanistic target of rapamycin (mTOR), which decreases mitotic activity and therefore has anti-tumor affects and influences mitochondrial homeostasis. Finally, downstream protein synthesis inhibition results in decreased production of both pro-inflammatory cytokines and reactive oxygen species (ROS) through activation of the thioredoxine antioxidant system decreasing DNA damage.15–18

In addition to its effects on human cellular mitochondria and systemic inflammation, metformin's direct effect on the gastrointestinal system has become an active area of research. Metformin has been shown to alter activity of the gastrointestinal endocrine system, increasing release of glucagon like peptide-1 (GLP-1) and pancreatic polypeptide (PPY) which regulate bile acids and control satiety and metabolism.20,21 Further, metformin controls and altering the abundance of specific bacteria and their response to stimuli.22 These alterations are thought to contribute to both glycemic control and the overall improved diabetic phenotype that results after administration of metformin. However, these positive effects have been minimally explored and are therefore not well understood in the non-diabetic patient.

Recently, there have been multiple prospective clinical trials exploring the effect metformin has in non-diabetic patients. Non-diabetic patients were treated with 250mg - 875mg three times daily or 2000mg daily for 14 days – 48 weeks showing an excellent safety profile. The metformin was very well tolerated with the primary side effect (30-60% of subjects) being self-limited gastrointestinal discomfort including mild diarrhea and anorexia.23–25 Across multiple disease states, metformin has been shown to decrease appetite stimulating GLP-1, decrease low density lipid profiles, decrease the mitotic rate in breast cancer, and improve liver pathology in patients with non-alcoholic steotatic hepatitis.1,12,20,23,25,26 As discussed above, some of these changes are attributed to the anti-glycemic and anti-tumor effects of metformin, yet the anti-inflammatory role cannot be underestimated. To our knowledge, there has been no study of the affect metformin has on inflammation, the microbiome, thrombosis, and cellular respiration as it links to physiologic and cognitive changes in non-diabetic patients.

Therefore, we hypothesize that aged non-diabetic subjects treated with a short course of metformin will have a beneficial change in metabolic and immune signaling and gut microbial homeostasis with an improved inflammatory response.

Study Objectives

This is a study examine if metformin improves the immune and inflammatory responses of non-diabetic, older adults.

We will begin to test this hypothesis by examining the following specific aims.
1. To determine the influence of metformin on basic metabolic, coagulation, and hematologic profiles

2. To discover the influence of metformin on physiologic and inflammatory responses in older adults including affects on peripheral blood mononuclear cells (PBMCs) phenotype and ex vivo inflammatory response to innate immune stimuli.
3. To discover the influence of metformin on thrombotic factors and platelet function in an ex vivo model.
4. To determine the influence of metformin on cellular respiration pathways including epigenetics and mitochondrial function.
5. Determine the influence of metformin on physiologic parameters of physical activity including grip strength as measured by a dynamometer and a short physical performance battery.
6. To establish dose specific gastrointestinal changes to the microbiome of metformin.

Study Design & Methods

All samples will be gathered prior to, throughout the 90 days (30, 60 and 90 days), and again approximately 30 days following completion of the metformin exposure.
1.) Changes in basic metabolic, coagulation, and hematologic profiles during and following exposure to metformin.
2.) Establish dose specific responses to affects on PBMC and ex vivo inflammatory response to innate immune system during and following metformin exposure. Cells will also be assessed for epigenetic changes by Chromatin immunoprecipitation.
3.) Understand the dose-specific effects of metformin on thrombotic factors, coagulation, and platelet function in an ex vivo model.
4.) Mitochondrial analysis of cellular respiration.
5.) Stool samples assessed for changes in the gastrointestinal microbiome

Cognition and physiology will be assessed at the baseline, 90 day, and 120 day time points.
6.) Physiologic testing: grip strength as measured by a dynamometer and a short physical performance battery

Unexpected adverse events including significant illness, hospitalization, emergent or urgent surgery or procedure, psychiatric hospitalization or inpatient rehabilitation, would require the study team to discontinue the subject's participation.

Additionally, if the subject fails to participate in the required study procedures they will receive compensation for those items that they have completed. However, they may be removed from the study and not allowed to participate in the study going forward. If participation needs to be discontinued, the study team will make up to 1-5 separate efforts to contact the subject in order to discuss their removal from the study and to instruct them to both discontinue and return the study medication. All unused study medication will be returned to the study pharmacy, IDS.

Eligibility Criteria

List the inclusion criteria:
1. Age ≥55 and ≤85 years of age
2. Non-diabetic
3. Adjusted risk analysis index (RAI) 20-42
4. Estimated glomerular filtration rate >=45
5. No evidence of hepatic dysfunction on comprehensive metabolic panel
6. No clinical evidence of cardiac failure
7. Existing University of Pittsburgh Medical Center Patients

List the exclusion criteria:
1. Hypersensitivity to metformin or any component of the formulation
2. Acute or chronic metabolic acidosis with or without coma
3. Pregnant or breastfeeding females
4. Evidence or history of hepatic, renal, or cardiac failure
5. Excessive acute or chronic ethanol use
6. Planned or known hospital admission, exposure to anesthesia, or surgical intervention 30 days prior to study or scheduled 30 days after the trial initiation
7. Laboratory analysis showing HbgA1c >6.1 or eGFR <45 on baseline labs

Statistical Considerations

This prospective randomized control study is powered based upon prior studies our group has completed on the cellular response to inflammation from PBMCs in healthy volunteers. This variable was chosen, as we believe this to be the most impactful variable included in this study. Previously, our group has looked at the immune response to lipopolysaccharide in healthy volunteers PBMCs ex vivo. In this study, we noted a statistically significant increase in pro-inflammatory cytokines between the two groups by approximately 40%. We expect a standard deviation of around 50% of the baseline values. Therefore, based upon previous inflammatory changes seen in healthy volunteers and a desired alpha = 0.05 and a beta = 0.2, our most conservative projected sample size using a two-sided Mann-Whitney test is 25 patients per group. With possible attrition we plan to enroll 30 patients per group. Therefore, across dosing we require a total of 120 patients in order to reach statistical significance. For this trial, we collecting data in order to pilot a larger trial after the we have data from this trial. Therefore, we expect to use the data from these 32 patients to understand how to power a larger study.

Patients will be used as their own controls before, during and after the exposure period, allowing for paired testing. Changes in laboratory venous and stool samples values will be compared using parametric and non-parametric standard statistics, as required. Differences in baseline physiologic testing will be controlled for based on their RAI score and the medical comorbidities, therefore each person serves as their own control. Both the 90 day treatment results and the 120 day test results (30 days after treatment discontinuation) for physiologic

testing will be compared to the individuals baseline values. Additionally, the various treatment doses will be compared at baseline, to ensure randomization allowed for equivalent patient groupings.

Randomization and blinding will be completed by IDS who will be provided with a block randomization scheme (see Sample Randomization in the Supporting Documents Section). The randomization scheme will have a block size of 8. Within each block, the randomization is classic and 1:1:1:1 between groups.

Changes from baseline will be investigated at each time point using ANOVA. Significance will be assumed with $p<0.05$.