Mito-LUTS: A Pilot Study of the Effect of MitoQ on Lower Urinary Tract Symptoms in Older Women with Metabolic Syndrome.

Protocol version 4.1
IND – 169630
IRB - #24-066-2
ClinicalTrials.gov – NCT06351683

PI: Iman M. Al-Naggar, PhD
Co-investigators: George Kuchel, MD, Peter C. Albertsen, MD
Collaborators: Jenna M. Bartley, PhD, Oh Sung Kwon, PhD

Funding/Sponsor: National Institute of Health / National Institute of Aging's Claude D. Pepper Older Americans Independence Center & UConn Health (P30 AG067988) **and** The Urology Care Foundation and American Urological Association New England Section Wyland F. Leadbetter, MD Award (Research Scholar Award)

Reviewed IRB Approved/Stamped Protocol

Investigator/Co-Investigator Name	Protocol Version	Date Reviewed	Signature
Iman M. Al-Naggar			
George A. Kuchel			
Peter C. Albertsen			
Jenna M. Bartley			
Oh Sung Kwon			

PROTOCOL VERSION # AND/OR DATE: Version 4.1 10/10/25

Title

Mito-LUTS: A Pilot Study of the Effect of MitoQ on Lower Urinary Tract Symptoms in Older Women with Metabolic Syndrome.

Background

Lower urinary tract symptoms (LUTS) have a high incidence, with well-documented negative effects on the quality of life of affected individuals. Symptoms including frequency, urgency, nocturia and incontinence are associated with an increased risk of falls, social isolation and lost independence [1]. Moreover, urinary incontinence can chip away at one of our most dearly held human values - dignity. In fact, in a recent study, 70% of hospitalized patients rated incontinence as an outcome equal to or worse than death[2].

Unfortunately, few people seek treatment and for those who do, drug options are limited, rarely beneficial and come with serious side effects, including cognitive dysfunction[3,4]. The mechanism of action of most bladder drugs (muscarinic receptor antagonists, β3-andrenergic receptor agonists) involves targeting the detrusor smooth muscle, which is often not the sole culprit. Novel medications targeting biological pathways involved in the pathophysiology of bladder dysfunction are urgently needed to alleviate LUTS.

Scientists studying diseases whose incidence increases with aging such as cardiovascular disease, cancer, diabetes and neurodegenerative disease, have observed the presence of common pathophysiologies. Varied biological mechanisms have been shown to play a role in aging and age- related disease, and have been collectively termed the 'Biological Hallmarks of Aging' (BHAs)[5]. BHAs include inflammation, fibrosis, telomere attrition, genomic instability, mitochondrial dysfunction, cellular senescence, lipotoxicity, nicotinamide adenine dinucleotide (NAD^+) depletion, oxidative stress, failed autophagy, stem cell exhaustion, loss of proteostasis, deregulated nutrient sensing, epigenetic alterations, and altered intercellular communication [5,6]. It was consequently hypothesized that interventions that delay biological aging pathways would also delay the onset of multiple age-related diseases (The Geroscience Hypothesis)[7]. Drugs targeting these BHAs have entered clinical trials for several aging-related diseases, with the goal of greatly reducing disease burden in older adults, thus increasing their healthspan (number of disease-free, independent years)[8]. The hope for these gerotherapeutics is to sharply reduce sickness and death from aging-related diseases as antibiotics once did for microbial infections. The goal of translational geroscience is not to make humans live indefinitely, but to help them remain healthy, functional and independent for the duration of their natural age.

Aging is a major risk factor for many diseases and syndromes[9]. Up to half of older adults experience LUTS [1], suggesting that they share common biological pathways with aging and other age-related diseases. Therefore, gerotherapeutics targeting BHAs may prevent or alleviate LUTS. The Metabolic Syndrome (MetS) is a condition characterized by glucose intolerance, obesity, hypertension and dyslipidemia that greatly increases the risk for cardiovascular diseases, including stroke and type 2 diabetes, and is often described as a state of "accelerated aging". In recent years, the MetS has also been recognized as a major risk factor for LUTS [10-12]. Inflammation, oxidative stress, mitochondrial dysfunction and deregulated nutrient sensing are some of the BHAs that have been implicated in LUTS pathology [13] (Figure 1). Other factors that increase with aging and are associated with both MetS and LUTS include ischemia and endothelial dysfunction [14].

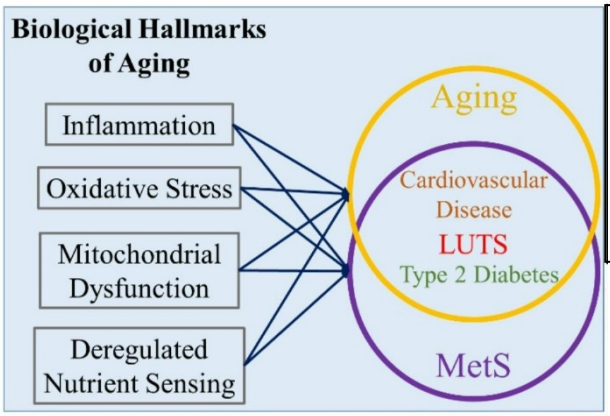

Figure 1: Aging and the metabolic syndrome share underlying biological mechanisms and are both risk factors for lower urinary tract symptoms. These shared biological mechanisms, or Biological Hallmarks of Aging, are targets of novel drugs called "gerotherapeutics.

Inflammation. Inflammation is an important homeostatic mechanism that becomes dysregulated with aging and plays a role in many chronic diseases and syndromes including diabetes mellitus, cardiovascular disease and frailty[15]. Unresolved inflammation can lead to cellular, tissue and ultimately organ damage through processes such as fibrosis and apoptosis. Increased inflammation in LUTS patients has also been reported; for example, inflammatory molecules such as C-reactive protein (CRP), nerve growth factor (NGF) and monocyte chemotactic protein-1 (MCP-1) increase in patients with overactive bladder syndrome (OAB), and correlate with advancing age [16-18]. Siddiqui et al. reviewed data for all biomarkers studies of LUTS, and although many technical and experimental issues were found to prevent confidence in biomarkers identified for clinical use, they found an association between immune and inflammatory pathways in OAB using pathway analysis[19]. Additionally, in the first non-targeted urine proteomics study carried out in LUTS patients for biomarker identification, Helfand *et al.* discovered that pathways enriched for in LUTS female patients included inflammation, dysregulated ion transport and immune response[20]. In rodent models, it was found that the NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome is upregulated with aging in urothelial cells, which leads to increased inflammation, fibrosis, urothelial remodeling and detrusor dysfunction[21]. It has been proposed that the NLRP3 inflammasome is activated in bladders in response to toxic materials in urine, hypoxia/reperfusion, increased pressure and repetitive stretching[22].

Oxidative Stress. Oxidative stress increases with both aging and MetS, with accumulating evidence strongly supporting its role in the pathophysiology and etiology of LUTS [23,24]. Oxidative stress is caused by disturbances in anti-oxidant regulation or increases in production of reactive oxygen species (ROS). Under normal conditions, ROS play an important role in cellular processes including autophagy and inflammation, cell growth and differentiation, metabolism and mitochondrial function. However, increased ROS beyond cellular homeostatic capacity leads to tissue and organ dysfunction via several possible mechanisms. For example, increased ROS lead to 1) increased secretion of inflammatory cytokines via activation of the Nuclear Factor κB (NFκB) and Adenosine monophosphate-activated protein kinase (AMPK) pathways, leading to the activation of pathological apoptosis and autophagy; and 2) increased macromolecular damage (to DNA, lipids and proteins) and accumulation of damaged molecules in cells via disruption of cellular machinery such as the ubiquitin-proteasome system, disrupting cellular processes. Dysfunctional mitochondria, which increase with aging and in metabolic conditions such as MetS and diabetes, are also a major source of ROS[25]. Mitochondrial ROS (mtROS) are main activators of the NLRP3 inflammasome, resulting in increased inflammatory cytokines including IL-1β and IL-18[26].

ROS can cause defects in detrusor myocytes, urothelium, organelles, vasculature and bladder innervation and cause cell death [24]. Ischemia and hypoxia, which can occur during bladder filling due to pelvic arterial atherosclerosis, can increase ROS and pelvic vascular insufficiency is believed to play a

role in the etiology of LUTS in older adults [27,28]. Smooth muscle hypersensitivity and increased contractile activity take place in organs such as the stomach, uterus and lungs when they become moderately ischemic and LUTS incidence correlates with peripheral arterial occlusive disease and decreased bladder blood flow in older adults [29,30]. ROS generated by repeated ischemia/reperfusion also leads to bladder denervation and fibrosis. Increases in endothelial ROS are associated with aging, metabolic and cardiovascular disease, and, of the many potential cellular sources of ROS in the vasculature, mitochondria are the major source [31,32]. Increased ROS in bladder epithelial cells of aged rats result in dysregulated mitochondrial bioenergetics, affecting barrier and sensory functions of the urothelium which are essential to proper function[33]. Drugs targeting ROS in aged rats, and antioxidants in a rat model of atherosclerosis-induced chronic bladder ischemia were shown to improve bladder function[34,35]. In humans, levels of urinary metabolites linked to mitochondrial dysfunction, oxidative stress and ketosis are reported to be higher in older female MetS patients with OAB, with potential use as biomarkers for OAB diagnosis and severity[36].

Thus, it is conceivable that restoring the redox balance, reducing inflammation and ensuing fibrosis and tissue damage, might alleviate or attenuate LUTS, and many such studies have been carried out in rodents [37]. MitoQ is a mitochondria-targeted anti-oxidant consisting of the naturally occurring anti-oxidant ubiquinol bound to a lipophilic cation (positively charged)[38]. This modification allows it to cross cell membranes and accumulate in the matrix facing the surface of the mitochondrial inner membrane where it is positioned for scavenging mitochondrial ROS and mimics the role of the endogenous mitochondrial antioxidant coenzyme Q10 (CoQ10) [38,39]. Additionally, MitoQ inhibits the NLRP3 inflammasome, which is well-established to play a role in the etiology of multiple bladder disorders[21,22]. This indirect inhibition of the NLRP3 inflammasome occurs as a consequence of the reduction in mtROS, restoration of mitochondrial integrity and membrane potential, and reducing mtDNA release (signal for NLRP3 inflammasome activation), causing a decrease in the pro-inflammatory cytokines IL-1β, IL-18 and TGF-β [40-42]. MitoQ also increases PINK1/parkin-mediated mitophagy of dysfunctional mitochondria, reducing ROS, inflammation and fibrosis[43,44]. Finally, MitoQ also improves vascular endothelial function by reducing mtROS and increasing nitric oxide (NO) availability in older adults and animal models[45-48]. We now hypothesize that these varied effects of MitoQ on ROS, mitochondrial health, inflammation and fibrosis, which affect all components of the urinary bladder (including detrusor, nerves, urothelium and vasculature) have the potential to alleviate or attenuate LUTS in older females with metabolic syndrome.

Hypotheses or Research Question, Aims and Objectives:

Hypothesis: Growing efforts to understand the pathophysiology of LUTS in aging and MetS have resulted in the identification of shared biological mechanisms, including inflammation, oxidative stress, mitochondrial dysfunction and metabolic imbalance. These varied mechanisms are collectively described as the biological hallmarks of aging and targeting them with "gerotherapeutics" is hypothesized to simultaneously delay or attenuate several aging-related diseases (The Geroscience Hypothesis). Gerotherapeutics have successfully been used in many pre-clinical models and evidence supports their use to prevent or attenuate LUTS, especially by targeting oxidative stress and inflammation. MitoQ is a strong anti-oxidant that can cross the mitochondrial membrane, improve mitochondrial health, decrease reactive oxygen species (ROS) and oxidative stress, increase nitric oxide bioavailability and reduce inflammation and fibrosis [39]. ***We hypothesize that MitoQ will attenuate MetS-associated LUTS in older women by targeting multiple shared biological hallmarks of aging.***

Question: Will lower urinary tract symptoms resulting from aging and metabolic syndrome in older women improve as a result of MitoQ supplementation?

Aims / objectives:

Objectives: The goal of this pilot study is to serve as proof-of-concept that using MitoQ, a supplement with gerotherapeutics properties, to target shared biological pathways between aging, metabolic syndrome and LUTS represents a much-needed novel direction in alleviating lower urinary tract symptoms. It will also help identify and validate biomarkers described in the literature for LUTS diagnosis and severity and generate data required to design and power future clinical trials.

Aims:

- **Aim 1: Elucidate the effect of MitoQ on lower urinary tract symptoms (LUTS) in older women with MetS.**

We will carry out a double-blinded, placebo controlled randomized pilot and exploratory study to test the effect of MitoQ on LUTS in older women with MetS. Older women (50years and older) with both LUTS (no UTI, with urgency possibly accompanied by other symptoms such as frequency, nocturia or urge incontinence for at least 3 months), and MetS (International Diabetes Federation (IDF) definition) will be randomized into a treatment (20mg/day MitoQ x1 week,40mg/day MitoQ thereafter, for 16 weeks, n=34) and placebo arm (n=16).
Assessments of LUTS using well-validated questionnaires and 3-day voiding diaries will be done at baseline, during, and at the end of the drug administration period. Main outcome will be change in LUTS questionnaire scores from baseline for each individual and between Placebo and MitoQ groups.
We hypothesize that treatment with MitoQ will improve LUTS questionnaire scores in women with MetS, whereas the placebo group scores will remain unchanged or worsen.

- **Aim 2: Measure the effect of MitoQ on biomarkers and their correlation with LUTS severity.**

There are currently no clinically useful biomarkers that are specific for LUTS, and novel biomarkers that can reliably distinguish LUTS are urgently needed. A good biomarker would also help with choice of therapy, predict response, change with an intervention and reflect changes in symptom severity.
Aim 2a: Does treatment with MitoQ in women with MetS-related LUTS alter blood and urinary biomarkers of biological hallmarks of aging and LUTS? We will measure biomarkers of inflammation and oxidative stress in blood and urine at baseline and 16 weeks after treatment initiation in our participants. We will also measure urinary proteins and metabolites shown to correlate with overactive bladder syndrome (OAB) [36,49] and others reported to be urotoxic or uroprotective[34]. Values will be compared to baseline for each subject and means will be compared between groups. *We hypothesize that MitoQ will reduce biomarkers of inflammation, oxidative stress, and OAB, while also decreasing urotoxic metabolites and increasing uroprotective metabolites.*
Aim 2b: Do changes in biomarkers correlate with types of LUTS and their severity? In order to be clinically useful, a biomarker should correlate well with LUTS type and severity. Biomarker levels will be correlated to LUTS questionnaire scores and voiding diary parameters. Because LUTS can have multifactorial etiology, no single biomarker has been useful. We aim to identify a panel of biomarkers for diagnosis, prognosis, tailoring, and tracking interventions. We hypothesize that changes in biomarkers will reflect changes in LUTS and correlate with LUTS severity.

Study Design:

This will be a randomized (1:2, placebo:MitoQ), placebo-controlled, double-blinded pilot and feasibility study. Participants in the MitoQ intervention group will receive MitoQ capsules (20mg/day x1 week, 40 mg/day thereafter), whereas placebo control group will receive capsules containing all inactive ingredients prepared by the same manufacturer without the MitoQ, for 16 weeks.

Sample size and justification: This being a pilot and feasibility study, a formal sample size calculation is not appropriate[50]. This study will serve many goals: 1) it will help us estimate standard deviation in our target population for the measures which will be used in a sample size calculation for future full-scale trials; 2) it will help us estimate the proportion of eligible people who are willing to participate, of participants who drop out of the trial, and of participants who comply with the proposed study protocol and intervention. However, a sample size justification is essential. Proposed sample sizes for pilot and feasibility range from 24, to 30 and 50 [51-53]. As sample sizes between 24 and 50 have been recommended for pilot and feasibility studies to help determine SD in parameters (such as the lower urinary tract symptoms questionnaire scores we will be assessing as outcome measures in this proposed study), we will use a sample size of 50. However, although as mentioned above a formal sample size calculation is not appropriate for the type of study proposed, we provide an example such calculation for Aim 1. Aim 1: Estimates of standard deviations (SD) for Overactive Bladder Symptom Score (OABSS) come from Chuang et al [54] who report SD in the range of 1.0 to 2.2 for patients with LUTS similar to our study. It is generally accepted that mean improvements in OABSS of 3 are clinically meaningful[55,56]. A sample size of 20 in each group will have 99% power to detect a difference in means of 3 assuming SD=2.2 using a two group t-test with a 0.05 two-sided significance level. Our planned analysis of covariance (ANCOVA) models should have even greater power characteristics than *t*-tests. Aim 2: We consider Aim 2 to be exploratory and supportive to Aim 1. No sample size calculations were done as the sample size was established at 50 based on recommendations for pilot and feasibility studies.

Explain on what basis it is reasonable to assume that the sample size will be obtained: Metabolic syndrome (MetS) is highly prevalent, affecting about 40% of Western populations[57]. Lower urinary tract symptoms are common in older adults and more so in people with MetS. An EPIC run Slicer Dicer Query identified 216 females between the ages of 50-75 with a history or diagnosis of metabolic syndrome with the ICD 10 code of 88.81 who were seen in the different UConn Health clinics between March 31, 2021 and March 31, 2023. We predict that a large portion of these older women with MetS will exhibit some LUTS, making them eligible for our study and helping us reach our sample size of 50.

Method(s) of data analysis: For this pilot study, will use a 1:2 randomization strategy, with twice as many people randomized to the study drug (MitoQ) than placebo. We will use stratified block randomization on BMI (<35 vs 35+) and age (<65 vs ≥65) to help control for potential confounding as subject number is modest. Randomization lists will be provided by a statistician. **AIM 1:** We will primarily use analysis of covariance (ANCOVA) to compare mean scores at week 16 between the placebo and MitoQ-treated group. Baseline values will be included as covariates. As the data set is small, perhaps one covariate such as age can be included. Repeated measures general linear models will be considered if the linear trends suggest an analysis with all timepoints is informative. Descriptive analyses will include paired *t*-tests for comparing LUTS questionnaire scores from Visits 2 and 3 with baseline (Visit 1), separately by group. Voiding diary measures (eg, number of trips to the bathroom during the day or at night, number of episodes of leakiness, number of urgency episodes) will be secondary outcomes and will be compared as done for questionnaire scores. We anticipate that the MitoQ-treated group will have a significant reduction in their LUTS severity (based on both questionnaire scores and voiding diary data) from baseline and compared to Placebo. **AIM 2:** Urinary

creatinine and osmolality will be used for urinary biomarker normalization and plasma/serum creatinine for blood biomarker normalization. The concentration for each biomarker will be compared for each subject at baseline and 16 weeks after treatment initiation using a paired t-test analysis. Additionally, post-treatment values for each biomarker will be compared between groups (placebo vs MitoQ) using an unpaired t-test. We will use the Benjamini–Hochberg method to control the False Discovery Rate (FDR) when presenting results of the t-tests. We anticipate to see a decrease in biomarkers of inflammation, oxidative stress and an increase in nitric oxide in the MitoQ group, with no changes in the placebo group. Biomarker concentrations will also be correlated to LUTS questionnaire scores and voiding diary parameters to identify biomarkers that correlate with LUTS severity.

Choice of MitoQ and Dose: MitoQ is a mitochondria-targeted anti-oxidant consisting of the naturally occurring anti-oxidant ubiquinol bound to a lipophilic cation (positively charged)[38]. This modification allows it to cross cell membranes and accumulate in the matrix facing the surface of the mitochondrial inner membrane where it is positioned for scavenging mitochondrial ROS and mimics the role of the endogenous mitochondrial antioxidant coenzyme Q10 (CoQ10) [38,39]. Additionally, MitoQ inhibits the NLRP3 inflammasome, which is well-established to play a role in the etiology of multiple bladder disorders[21,22]. This indirect inhibition of the NLRP3 inflammasome occurs as a consequence of the reduction in mitochondrial Reactive Oxygen Species (mtROS), restoration of mitochondrial integrity and membrane potential, and reducing mitochondrial DNA release (signal for NLRP3 inflammasome activation), causing a decrease in the pro-inflammatory cytokines IL-1β, IL-18 and TGF-β [40-42]. MitoQ also increases PINK1/parkin-mediated mitophagy of dysfunctional mitochondria, reducing ROS, inflammation, and fibrosis[43,44]. Finally, MitoQ also improves vascular endothelial function by reducing mtROS and increasing nitric oxide (NO) availability in older adults and animal models[45-48]. We now hypothesize that these varied effects of MitoQ on ROS, mitochondrial health, inflammation and fibrosis, which affect all components of the urinary bladder (including detrusor, nerves, urothelium and vasculature) have the potential to alleviate or attenuate LUTS in older women with metabolic syndrome.

We chose the MitoQ formulation because in a comprehensive pharmacokinetic assessment, it provided superior achievement of maximum blood antioxidant concentrations. A daily dose of 40mg was chosen because it is reported by the maker of MitoQ to be beneficial without side effects. Doses up to 80mg have been tested in multiple human studies, all with no safety concerns[58,59].

Study Procedures

Please refer to Schedule of Events (Table 1) for details of each study visit. Study design timeline is illustrated in Figure 2. Potential participants will be phone screened for selected inclusion and exclusion criteria (outlined in Enrollment section) prior to scheduling the in-person screening visit. If potential participants meet selected inclusion and exclusion criteria, they will be scheduled for a Screening Visit at the Center on Aging clinical research center within 4 weeks. During the screening visit, they will have the study explained to them and if they choose to participate, they will sign the Informed Consent Form (ICF) and HIPAA waiver. Medical history and medication history will be obtained, Metabolic syndrome, LUTS, cognition, physical and frailty assessments will be performed, a urine sample collected for urinalysis and a blood sample will be drawn and then analyzed for glycated hemoglobin (HgA1c), triglycerides, cholesterol, serum creatinine, and ALT/AST. An electrocardiogram test (ECG) will also be done. Once results from laboratory tests are received, eligibility for the study via full inclusion and exclusion criteria will be determined and eligible subjects will be scheduled for their baseline visit (Visit 1), no later than 4 weeks from the Screening Visit. At the baseline visit (week 0, Visit 1), following the completion of study assessments, subjects will be randomized and receive their respective medication, MitoQ or placebo. Drug

double-blinding and dispensing will be done by the UConn Health Pharmacy. Recruitment and screening will take place approximately April 2024 to July 2025. We envision all study visits to be completed by December, 2025. We anticipate up to 22 weeks of participation from screening visit to study end, including the 16-week study drug intervention time. All study visits will occur in the Center on Aging clinical research center that is suitable for clinical testing.

- *Medical History:* A detailed medical history will be obtained from all participants at the in-person screening visit to rule out any advanced chronic diseases and confirm full study eligibility.
- *LUTS Questionnaires:* Several validated LUTS questionnaires will be administered at screening and at Visits 1-3 and Phone Calls A and B. For screening of LUTS, with urgency being the main symptom, we will use the Urgency Perception Scale [60], and the Homma et al. Overactive Bladder Symptom Score questionnaires (OABSS)[61]. Inclusion criteria for LUTS are having urgency with or without other urinary symptoms for at least 3 months, with a score of 1 or 2 on the Urgency Perception Scale questionnaire [60], and a total score of at least 6on the OABSS questionnaire (with at least a score of 2 on the third OABSS question "How often do you have a sudden desire to urinate, which is difficult to defer?"). Questionnaire scores will be the main outcome measures for assessing the effect of MitoQ on LUTS. Questionnaires used will include 1) The International Consultation on Incontinence Questionnaire Female Lower Urinary Tract Symptoms Long Form Module (ICIQ-FLUTS LF) for screening for urinary symptoms and impact on quality of life [62], 2) The Overactive Bladder Symptom Score (OABSS)[61,63], 3) The Symptoms of Lower Urinary Tract Dysfunction Research Network Symptom Index 29 (LURN SI-29)[64], 4) The Overactive Bladder Questionnaire (OAB-q)[65-68] and 5) the Urgency Perception Score [69]. The Urgency Perception Score and the OABSS will also be administered by phone during Phone Calls A and B at 4 and 12 weeks. Changes in the OABSS will serve as our primary outcome with a decrease of 3 points or more considered to be clinically meaningful.
- *Voiding Diaries*: Three-day voiding diaries (consecutive or non-consecutive) will be requested at each study visit. Subjects will be instructed on how to prepare them using the Urology Foundation template and instructions, copies of which will be provided for each visit. The use of voiding diaries is routine in clinical practice to assess accuracy of reported urinary symptoms.
- *Measure of Cognitive function:* A questionnaire to measure cognitive status will be done at the in-person screening visit to confirm eligibility for the study and repeated at Visit 3 to check for any changes. We will use the Telephone Interview for Cognitive Status-Modified (TICS-M)[70] 13-question questionnaire to assess cognitive function and confirm eligibility for study inclusion during the in-person screening visit. The TICS-M Scores can range from 0 to 50 and participants who score 30 or less on the TICS-M questionnaire will be excluded from the study[71,72]. Participants scoring between 31 and 34 will be noted as possibly having mild cognitive impairment (MCI) but will be eligible.
- *MetS assessment*: International Diabetes Federation (IDF) definition of MetS will be used to determine MetS status and eligibility for inclusion[73]. Blood pressure, height, weight, waist circumference, fasting glucose, hemoglobin A1c (HgA1c), and blood triglyceride/cholesterol will be measured at screening to determine metabolic syndrome status. MetS will be defined as having central obesity with ethnicity-specific values, plus two or more of the following criteria: Hypertension, dyslipidemia, or hyperglycemia[73]. Hypertension is defined by the use of anihypertensive medication and/or blood pressure of ≥130/85 mmHg. Dyslipidemia is defined as blood triglycerides >150mg/dL or high-density lipoprotein (HDL) < 50mg/dL. Hyperglycemia is defined as a fasting glucose ≥100mg/dL. The ethnicity-specific cut offs we will use are as follows:

Asian women: ≥80cm[74], White women of European origin: ≥88cm[75], Black women, Sub-Saharan Africa: ≥94cm[76], Latin American: ≥90cm[77]. John Dempsey Hospital (JDH) laboratory at UConn Health will carry out blood analysis consisting of 1) a standard lipid panel, 2) HgA1c, and 3) comprehensive metabolic panel for the above assessments. Samples will be transported per appropriate protocols to labs. These assessments will be repeated at the final visit (Visit 3).

- *Safety Labs/Blood Chemistries:* For safety monitoring of participants, blood will be collected at screening, Visit 2 and Visit 3 and sent to JDH laboratory for 1) complete blood count (CBC) with differential and 2) comprehensive metabolic panel (CMP) analyses. Samples will be transported per appropriate protocols to labs. In order to properly analyze lab results relative to age-appropriate normal value ranges and ensure results obtained correspond to the correct study participant, blood samples sent to JDH laboratory for processing will be labeled with participant research medical record ID (TO#), name and Date of Birth (DOB). The DOB, TO# and name will then be redacted in printed copies of test results kept in participant binders and replaced with participant Study ID.
- *Medication Adherence*: In order to assess subject adherence to the intervention, they will be requested to bring unused pills at Visits 2 and 3 and pills will be counted and recorded.
- *Blood Collection:* Fasting blood samples will be obtained by standard venipuncture at screening and all study visits for biomarker analysis and safety labs/blood chemistries. After processing, serum and plasma will be aliquoted and stored at -80°C until analyzed. A finger prick for fasting blood glucose determination using a glucometer will also be done at the beginning of each visit. Samples will be transported per appropriate protocols to labs.
- *Urine Collection*: Subjects will be given urine collection cups for first morning urine collection and instructed to keep them frozen until brought in for analysis at Visits 1-3. Twenty-four hour urine collections will also be requested ahead of visits 1-3 and participants given instructions on proper collection and storage. Participants will be given the option of opting out of the 24-hour urine collections at the beginning of the study if they feel it may be a barrier to participation or is too burdensome. All materials will be given to or mailed to participants in advance. Two spot urine samples will be collected at each visit (fasting upon arrival and a second sample at the end of the visit (may or may not be fasting). Some spot urine will be used for urinalysis. After centrifugation, remaining spot urine will be aliquoted and stored at -80°C until analyzed.
- *Frailty Measures:* The Frailty Index is beneficial to detect change over time, whereas the Fried Model is used to diagnose the Frailty phenotype. The frailty phenotype will be assessed at the screening visit for exclusion of frail individuals using the Fried Model. The Rockwood Frailty Index will be done at visits 1 and 3.

  Fried Model: In Fried's model, the presence of three or more of five indicators (unintended weight loss, tiredness, weak grip strength, slow walking speed, and physical inactivity) predicts a range of adverse outcomes including falls, disability, admission to hospital and death. We will use the Physical Activity Scale for the Elderly (PASE) measure to assess tiredness and physical activity/inactivity.

  Rockwood Frailty Index: The Frailty Index (FI) uses data on co-morbidity, function and health practices. Items to be included in the frailty index are as follows: (1) each item will be a health deficit, by which we mean it is associated with adverse outcomes, increases with age and does not saturate (i.e. is not present in everyone); and, (2) deficits (which can be diseases, disabilities, symptoms, or laboratory abnormalities) will each be coded so that 0 will represent the absence of the deficit and 1= the presence of the deficit. We expect to have at least 30 deficits, which appears to be enough to allow for the Frailty Index to show characteristic properties. For any individual, their frailty Index score would be the number of deficits that they have, divided by

the total considered – e.g., if we have 40 items, a person with 8 deficits would have an FI of 8/40 = 0.20). In general, people with a low level of frailty (FI < 0.05) are fit, whereas those with an FI > 0.4 are frail[78]. The value of the FI is closely correlated with a variety of adverse outcomes, including mortality and health service use. The method is very robust and has been multiply cross-validated as linking to shorter and longer term adverse outcomes in a variety of population and clinical settings.

- *Physical Mobility Assessments:* The Short Physical Performance Battery (SPPB) is an objective assessment tool for evaluating lower extremity functioning in older persons. It was developed by the National Institute on Aging. The test includes three different domains (walking, sit-to-stand and balance) to assess functional mobility. Trained research staff will administer the SPPB to participants at screening and at Visit 3.

- *Pregnancy Screening:* Women who are pregnant will be excluded from study participation. Pregnancy status will be inquired about during the screening call and pregnant women will be excluded. Furthermore, we will administer urine pregnancy tests to all women who have had a menstruation within the last 12 months. Only women whose pregnancy tests are negative will be invited to participate in the study.

	Phone Screen	Screening Visit	*Week 0* Visit 1	*Week 4* Phone Call A	*Week 8* Visit 2	*Week 12* Phone Call B	*Week 16* Visit 3
Consent/HIPAA		X					
Randomization			X				
Demographics		X					
Medical History		X					
Medication History		X					
Vitals and Anthropometrics		X	X		X		X
Medication Dispensed			X		X		
Medication Counted					X		X
Urgency Perception Scale	X						
LURN-SI29			X		X		X
Urgency Perception Score		X	X	X	X	X	X
Overactive Bladder Symptom Score	X	X	X	X	X	X	X
ICIQ-FLUTS LF			X		X		X
OAB-q			X		X		X
SPPB		X					X
PASE		X					
Fried Frailty Phenotype		X					
Rockwood Frailty Index			X				X
Modified TICS (cognitive screening)		X					X
Pre-treatment TAAS		X					
Post-treatment TAAS							X
Mito-LUTS Participant Feedback Form							X
Electrocardiogram		X			X		X
Finger stick (fasting blood glucose)		X	X		X		X
Blood draw		X	X		X		X
HgA1c		X					X
Comprehensive Metabolic Panel		X			X		X
Complete Blood Count with Differential		X			X		X
Standard lipid panel		X					X
24-hour urine and first morning urine			X		X		X
Spot urine X2		X	X		X		X
Urinalysis		X	X		X		X
3-Day Voiding Diary			X		X		X
Pregnancy Test		X					
Adverse Events				X	X	X	X
Medication Update			X	X	X	X	X
Adherence				X	X	X	X
Medical History Update			X	X	X	X	X
Instructions (diary and urine collections)		X	X		X		
Inclusion/Exclusion	X	X					

Table 1: Schedule of Events

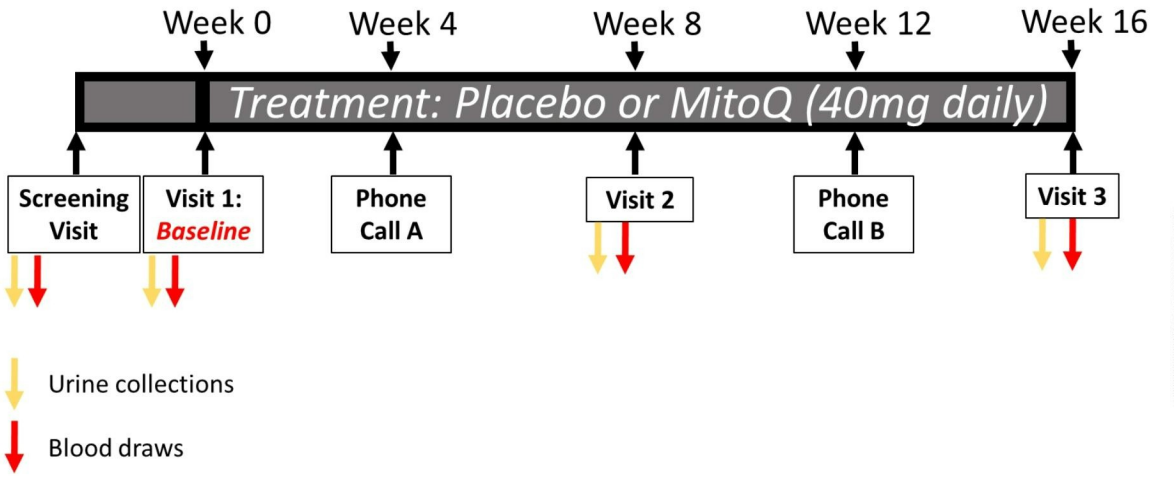

Figure 2: Timeline of Study Visits and Assessment Calls (Initial and bi-weekly check in calls and visit reminder calls not shown)

Study Events

Phone Screen

The study will be advertised using IRB-approved flyers that will be posted and placed in waiting rooms and clinics (e.g., UConn emergency department, internal medicine, family medicine, geriatric medicine, UConn Urgent Care in Canton, etc.) throughout UConn Health and community centers and other public places throughout Connecticut to recruit individuals. Patients will also be identified through daily EPIC reports from the UConn Health outpatient clinics, based on initial screen based on sex, age and ICD10 for metabolic syndrome (E88.81). Patient's physician or clinical provider will seek verbal assent from patients for referral to UConn Center on Aging. Patients will be contacted by our study research coordinator as to their potential interest in participating in the study. Interested subjects will call or email to inquire about the study. Potential subjects will be phone screened prior to scheduling the screening visit to determine if they meet specific demographic and inclusion/exclusion criteria. They will answer questionnaires to assess for the presence of the metabolic syndrome and lower urinary tract symptoms (the Urgency Perception Scale [60], and the Overactive Bladder Symptom Score (OABSS). Potentially eligible participants will be scheduled for a screening visit. (~30 minutes)

A Post-Phone Screening Call Eligibility Checklist Review will be done by PI or research staff with the primary clinical/medical investigator on the study or study nurse to ensure preliminary eligibility before inviting volunteer to in-person screening visit.

Screening Visit

Screening visits will be scheduled within 4 weeks of phone screening. Participants will be asked to arrive following an overnight fast. During the screening visit, potential subjects will have the study explained to them and if they choose to, sign the ICF and HIPAA waiver. After signing ICF and HIPAA waiver, the pre-treatment "Treatment Acceptability and Adherence Scale" (TAAS) questionnaire will be administered. Vitals and anthropometrics (temperature, heart rate, blood pressure, waist circumference, height, and weight) will be measured. A fasting blood glucose finger prick measure with a glucometer will be done and we will collect fasting urine and blood samples (via standard venipuncture). Blood will be sent to a lab for analysis of glycated hemoglobin (HgA1c), comprehensive metabolic panel, complete blood count with differential and lipid panel. Urinalysis will be performed on spot urine to confirm absence of active Urinary Tract Infections (UTIs). Women will be asked about their menopause status, and we will administer a pregnancy test to any woman who has had a menstruation in the past 12

months to confirm she is not pregnant. Participants will be offered a light breakfast. Demographics, complete medical history and medication history will be obtained. LUTS questionnaires (OABSS and Urgency Perception Score [69]), Short Performance Physical Battery (SPPB) and other assessments of frailty (Hand grip test, PASE, Fried Frailty Phenotype [79,80]) and cognition (M-TICS) questionnaires will also be included for screening. ECG measurement will be done to determine QTc interval for eligibility. A second spot urine sample will be collected at the end of the visit. In order to confirm full eligibility and obtain information about any medical conditions that may affect the outcome of the study, the PI or study coordinator will access the participant's UConn Health medical record and look for information related to their overall health and any urological, gynecological, gastrointestinal and metabolic conditions. We will give participants instructions on urine sample collection and storage (first morning urine, 24-hour urine) and give instructions for the 3-day bladder diary. Once results from laboratory tests are received, eligibility for the study via full inclusion and exclusion criteria will be determined and eligible subjects will be contacted to schedule their Visit 1 (baseline). (~2.5 hours, $25 check)

A Post-In Person Screening Visit Eligibility Checklist Review will be completed by PI or research staff with the primary clinical/medical investigator on the study, Dr. Kuchel, or study nurse to ensure FULL eligibility to participate in the Mito-LUTS study. Results of ECG and blood tests (Standard lipid panel, Hemoglobin A1c, Comprehensive metabolic panel (CMP) and complete blood count (CBC) with differential and platelets must have been obtained before reviewing this checklist.

Visit 1 (Week 0, Baseline)

Visit 1 will be scheduled within 4 weeks of in-person screening visit. Participants will be instructed to arrive with a filled out 3-day voiding diary, previous day's frozen first morning urine and optional 24-hour urine collection which we will collect upon arrival. Instructions and materials required for all urine collection will either be provided to participants at the end of the screening visit or mailed to their homes after eligibility is confirmed. At the baseline visit participants will arrive to the Center on Aging (CoA) following an overnight fast (with the exception of water). Vitals and anthropometrics (temperature, heart rate, blood pressure, waist circumference, height, and weight) will be measured by trained research staff. A fasting blood glucose finger prick measure with a glucometer will be done and we will collect fasting urine and blood samples (via standard venipuncture by trained research staff). Urinalysis will be performed on spot urine to confirm absence of active Urinary Tract Infections (UTIs). Participants will be offered a light breakfast. Medical and medication history will be updated. Bladder diary will be reviewed with participant. Study staff will administer the LUTS questionnaires to the participant (ICIQ-FLUTS, OABSS, OAB-q, Urgency Perception Score and LURN-29). We will administer the Rockwood frailty index questionnaire and measures to participants. We will review instructions for the first morning, 24 hour (optional) and spot urine collection and storage guidelines with participants and provide them with instructions and collection materials to take home. We will also review instructions on the 3-day voiding diary with participants. Participants will be instructed on when and how to take the capsules and also on the "Lean Forward Method" for taking capsules and watch a short video (https://www.youtube.com/watch?v=2Czc_oLg8QA). Subjects will then be randomized to their treatment assignment and will receive study drug. Participants will be instructed to take one capsule per day for the first week, preferably 30 minutes before breakfast, then increase to two capsule per day from Week 2 onwards. A second spot urine sample will be collected at the end of the visit. When possible, we will schedule twice a week phone calls for the first 3 weeks or contact participant at a later time to schedule them. Participants will receive a $50 check. (~1 hour)

Phone Call A (Week 4, Window: between week 4-6 of drug initiation)

Research staff will perform calls to study participants to administer LUTS questionnaires over the phone (OABSS, Urgency Perception Score questionnaires). Adherence and adverse effects will also be inquired about during this call. Medical history and medication updates will take place. (~10 minutes)

Visit 2 (Week 8, Window: between week 8-10 of drug initiation)

Visit 2 will be scheduled 8 weeks from Visit 1 (no later than 10 weeks from Visit 1). Participants will be instructed to arrive fasting, with a filled out 3-day voiding diary, previous day's frozen first morning urine, and optional 24-hour urine collection which we will collect upon arrival. Study staff will collect and document remaining medication. Vitals and anthropometrics (temperature, heart rate, blood pressure, waist circumference, height, and weight) will be measured by trained research staff. A fasting blood glucose finger prick measure with a glucometer will be done and we will collect fasting urine and blood samples (via standard venipuncture by trained research staff). Blood will be sent to a lab for comprehensive metabolic panel and complete blood count with differential (for safety labs and biomarker analysis). Urinalysis will be performed on spot urine to confirm absence of active Urinary Tract Infections (UTIs). Participants will be offered a light breakfast. Medical and medication history will be updated and any side effects, symptoms or AEs will be documented and addressed. Bladder diary will be reviewed with participant. Study staff will administer the LUTS questionnaires to participants (ICIQ-FLUTS, OABSS, OAB-q, Urgency Perception Score and LURN-29). ECG measurement will be done to determine QTc interval for safety. We will review instructions for the first morning, 24 hour (optional) and spot urine collection and storage guidelines with participants and provide them with instructions and collection materials to take home. We will also review instructions on the 3-day voiding diary with participants and the "Lean Forward Method" for taking capsules. Subjects will receive study drug for remaining 8 weeks. A second spot urine sample will be collected at the end of the visit. We will schedule Week 10 and Week 12 phone calls during this visit when possible, otherwise participants will be contacted later for scheduling. Participants will receive a $75 check. (~1.5 hours)

A Post-Visit 2 Safety Checklist Review will be done by PI or research staff with the primary clinical/medical investigator on the study, Dr. Kuchel, or study nurse to ensure participant safety and continued eligibility to stay in the study. Results of ECG and blood tests (Comprehensive metabolic panel (CMP) and complete blood count (CBC) with differential) must have been obtained before reviewing this checklist.

Phone Call B (Week 12, Window: between week 12-14 of drug initiation)

Research staff will perform calls to study participants to administer LUTS questionnaires over the phone (OABSS, Urgency Perception Score questionnaires). Adherence and adverse events will also be inquired about during this call. Medical history and medication updates. (~10 minutes)

Visit 3 (Week 16, Window: between week 16-18 of drug initiation)

Visit 3 will be scheduled 16 weeks from Visit 1 (no later than 18 weeks from Visit 1). Participants will be instructed to arrive fasting, with a filled out 3-day voiding diary, previous day's frozen first morning urine, and optional 24-hour urine collection which we will collect upon arrival. Study staff will collect and document remaining medication. Vitals and anthropometrics (temperature, heart rate, blood pressure, waist circumference, height, and weight) will be measured by trained research staff. A fasting blood glucose finger prick measure with a glucometer will be done and we will collect fasting urine and blood samples (via standard venipuncture by trained research staff). Blood will be sent to a lab for analysis of glycated hemoglobin (HgA1c), comprehensive metabolic panel, complete blood count with differential and lipid panel (for safety labs and biomarker analysis). Urinalysis will be performed on spot urine to confirm absence of active Urinary Tract Infections (UTIs). Participants will be offered a light breakfast. Medical and medication history will be updated and any side effects, symptoms or AEs will be documented and addressed. Bladder diary will be reviewed with participant. Study staff will administer the LUTS questionnaires to participants (ICIQ-FLUTS, OABSS, OAB-q, Urgency Perception Score and LURN-29). ECG measurement will be done to determine QTc interval for safety. Participants will complete the Short Performance Physical Battery (SPPB) and other assessments of frailty (Hand grip test, and the Rockwood Frailty Index [79,80]) and a cognition questionnaire (M-TICS). A second spot urine sample will be collected at the end of the visit. The post-treatment "Treatment Acceptability and Adherence Scale" (TAAS) and the Mito-LUTS Participant Feedback Form questionnaires will be administered. Participants will receive a $100 check. (~2.5 hours)

A Post-Visit 3 Safety Checklist Review will be done by PI or research staff with the primary clinical/medical investigator on the study, Dr. Kuchel, or study nurse to ensure participant safety. Results of ECG and blood tests (Comprehensive metabolic panel (CMP) and complete blood count (CBC) with differential) must have been obtained before reviewing this checklist.

Other Phone Calls

During the first three weeks following Visit 1, the subjects will receive twice weekly phone calls to remind them to take their medication and inquire about any adverse events. Every two-week phone calls will then be performed to check for any potential medication side effects and encourage adherence. Reminder calls 4-7 days prior to Visits 1, 2 and 3 will also be used to review 3-day voiding diary, first morning and optional 24-hour urine collection and storage instructions. (~5 minutes)

Other Study Components

Missed medication doses

Participants will be advised to take the medication at the same time every day. If they forget to take the medication at that time, but remember later in the day, they will be instructed to take the medication when they remember that day. If participants forget to take the medication for a full day, they will not be advised to double medication for the following day, but rather to just resume taking their medication as normal, e.g. once daily, and report the missed dose to the research staff during their phone calls and medication adherence checks.

Rationale for fasting blood draws and urine samples

Participants will be asked to fast overnight for the screening visit and visits 1-3 to examine certain metabolic parameters that may be influenced by prandial state, specifically blood glucose (as part of the comprehensive metabolic panel performed for clinical assessments), as well as circulating IL-6 and other factors. These additional metabolic parameters will be examined in serum/plasma/urine collected from the participants from baseline visit 1 to visit 3 (pre to end of treatment) and require fasting for accurate interpretation. Thus, participants will be asked to fast overnight prior to these visits.

Laboratory Tests

Serum/Plasma Isolation

Serum will be isolated from a vacutainer with no additive (4mL), while plasma will be isolated from an EDTA vacutainer (4mL). Measures of aging and inflammatory biomarkers, oxidative stress, nitric oxide and other LUTS-associated biomarkers will be made in the UConn Pepper Center Biomarkers Core (RC3) and the Facility for Geroscience Analysis at the Mayo Clinic (Minnesota) using established methods.

A 5mL serum separator tube (SST) and 1-2 4mL EDTA tubes for analysis of the comprehensive metabolic panel and standard lipid panel, CBC and HbA1c, which are required for clinical safety testing and metabolic syndrome status determination.

Urinalysis

Urinalysis will be performed on urine samples collected using a dipstick. Specific gravity will be measured using a refractometer to determine sample hydration status. Urine will be processed and frozen for biomarker analysis and creatinine measurement.

Routine Blood Tests and Chemistries

JDH Laboratory at UConn Health will perform routine blood chemistries and clinical laboratory assessments for inclusion and safety. The specific tests are indicated below.

Test	CPT Code	Specimen Type	Minimum Quantity	Temp for transport	When?
HbA1c	83036	Whole blood, EDTA lavender top tube	1.0mL, prefer 4mL lavender tube	RT	Screening, Visit 3
Complete Blood Count with Differential	85025	Whole blood, EDTA lavender top tube	0.5mL, prefer 4mL tube	RT	Screening, Visit 2 and Visit 3
Comprehensive Metabolic Panel	80053	Serum (SST tube)	1.0mL, prefer 5mL SST	RT	Screening, Visit 2 and Visit 3
Standard Lipid Panel	80061	Serum (SST tube)	1.0mL, prefer 5mL SST	RT	Screening, Visit 3

Sample Collections

Blood Collection Per Visit

Blood collection tubes used per visit are listed below.

Tube	Test	Screening	Visit 1	Visit 2	Visit 3
4 mL EDTA tube	CBC w/diff	X		X	X
4 mL EDTA tube	HbA1c	X			X
5mL gold top SST tube	CMP and Lipid Panel	X		X	X
4mL gold top SST tube (3)	Serum Isolation	X	X	X	X
4mL lavender top EDTA tube (3)	Plasma Isolation	X	X	X	X
Total blood volume drawn per visit (mL):		37	24	33	37
Total blood volume drawn in the study (mL):		**131**			

Urine Collection Per Visit

Urine collections per visit are listed below.

Sample	Volume	Screening	Visit 1	Visit 2	Visit 3
24-hour urine collection (optional)	Variable (1-5L)		X	X	X
Spot urine sample (fasting)	60mL	X	X	X	X
Spot urine sample (non-fasting)	60mL	X	X	X	X
First morning urine sample	60mL		X	X	X

Subject Characteristics

Overview

Women aged 50 or older diagnosed with metabolic syndrome (ICD 10 code E88.81) or fulfilling IDF criteria for metabolic syndrome recruited at UConn Health or elsewhere will be invited to complete an IRB-approved preliminary screening questionnaire over the phone to pre-screen subjects for key eligibility criteria (demographics, Metabolic syndrome and LUTS diagnoses). A screening visit will occur to review all medical history and confirm initial eligibility criteria. Blood for serum creatinine, triglycerides, cholesterol, fasting glucose, AST/ALT, and HbA1c lab work will be obtained to determine full eligibility. ECGs will also be performed at the screening visit to measure QT interval. Eligible participants will be scheduled for a baseline visit. At the baseline visit (Visit 1) medical history will be updated and baseline measures will be collected. Participants will be randomized (double blinded) and receive their assigned study medication.

Participant Inclusion/Exclusion Criteria

Inclusion Criteria

The new IDF's 2006 consensus worldwide definition for MetS will be followed for inclusion[73]. MetS will be defined as having central obesity with ethnicity-specific values, plus two or more of the following criteria: Hypertension, dyslipidemia, or hyperglycemia[73]. Hypertension is defined by the use of antihypertensive medication and/or blood pressure of ≥130/85 mmHg. Dyslipidemia is defined as blood triglycerides >150mg/dL or high-density lipoprotein (HDL) < 50mg/dL, or specific treatment for either lipid abnormality. Hyperglycemia is defined as a fasting glucose ≥100mg/dL. Inclusion criteria for LUTS are having urgency with or without other urinary symptoms for at least 3 months, with a score of 1 or 2 on the Urgency Perception Scale questionnaire [60] , and a total score of at least 6 on the OABSS questionnaire (with at least a score of 2 on the third OABSS question "How often do you have a sudden desire to urinate, which is difficult to defer?").

- Women 50years or older with metabolic syndrome and LUTS as defined above.
- Speak, read and understand English
- Willingness to provide consent and participate in all aspects of the trial including randomization to the intervention group

Exclusion Criteria

The exclusion criteria for Mito-LUTS include conditions that will interfere with our research questions, including advanced co-morbidities and immunological disorders. They are as follows:

- Frailty, defined as meeting 3 of 5 frailty indicators of the Fried Frailty Phenotype
- History of severe renal impairment and/or eGFR ≤ 60 mL/min/1.73m^2 at the study physician's discretion
- Excessive alcohol use (more than 14 alcoholic drinks/week)
- Clinical/laboratory evidence of hepatic disease (via medical history and/or AST and/or ALT ≥ 3 times upper limit of normal at screening)
- Poorly Controlled Diabetes
- Unwilling or unable (due to significant cognitive impairment) to provide informed consent.
- Terminal illness with life expectancy less than 12 months
- Advanced neurological disorder (Alzheimer's, Parkinson's, ALS, MS, dementia, seizures)
- A score of 30 or less on the modified Telephone Screening of Cognitive Status administered during the in-person screening visit.
- Cancer or history of gynecological cancer or history of cancer requiring chemotherapy or radiation at the study physician's discretion.
- A history of gastric ulcers.
- Abnormal findings on endoscopy.
- Recent (within the last 2 weeks) or current chronic use of NSAIDs or other drugs or agents with the potential for gastric mucosal toxicity (except for daily use of baby aspirin or famotidine for which participants will not be excluded). Sporadic use of NSAIDs will not be an exclusion

criterion.

- Significant co-morbid disease (severe chronic obstructive pulmonary disease, active rheumatologic diseases, chronic infection (HIV, tuberculosis), severe congestive heart failure (NYHA class 4), myocarditis, etc)
- Myocardial infarction, stroke or hospitalization for heart failure in the last 12 months
- QTc >460 ms at screening on ECG
- Prior diagnosis of ventricular arrhythmia (e.g., ventricular tachycardia, ventricular fibrillation, torsades de pointes)
- Severe active psychiatric disorder (e.g. bipolar, schizophrenia)
- Unable to complete physical performance testing due to medical conditions (at discretion of the PI)
- Unintentional weight loss >15 lbs in past 12 months
- Immunosuppressive disorders or taking immunosuppressive medications (including oral prednisone > 10mg/day)
- Sub-cerebellar lesions
- Subjects must not be on warfarin or other blood thinning medications or have a known bleeding disorder.
- Conditions that might interfere with clinical diagnosis (such as pelvic organ prolapse ≥ stage 2, pelvic radiotherapy, any concurrent condition that could cause incontinence, hematuria, vaginitis, neurogenic lower urinary tract dysfunction); chronic pelvic pain syndrome, interstitial cystitis/bladder pain syndrome, pelvic malignancy, active urinary tract infection (UTI), recent urologic procedure (<6 months).
- Clean intermittent catheterization or indwelling catheter
- Current participation in another interventional study
- Pregnancy and nursing
- Subjects must not have used antibiotics for at least 3 weeks prior to visit 1, received a vaccination in the 2 weeks prior to visit 1 or used medicine that alters the immune response (eg high dose corticosteroids) in the 6 months prior to visit 1. Subjects must not have had an acute infection in the 3 weeks prior to visit 1 or had a major severe illness or been hospitalized in the 3 months prior to visit 1. If participants are within any of these windows when they are screened, they will be scheduled for a screening visit but will only be invited for Visit 1 after these specified windows have elapsed and/or infections have resolved.
- Subjects must not be on or have taken any of the following anti-muscarinics or β3-adrenoreceptor agonists for 3 weeks prior to visit 1:
    - Darifenacin (enablex)
    - Oxybutynin (ditropan)
    - Solifenacin (vesicare)
    - Fesoterodine (Toviaz)
    - Tolterodine (detrol)
    - Trospium (sanctura)
    - Imipramine (tofranil)
    - Mirabegron (myrbetriq)
    - Vibegron (gemtesa)

Recruitment

Recruitment

An EPIC run Slicer Dicer Query identified 216 women between the ages of 50-75 with a history or diagnosis of metabolic syndrome with the ICD 10 code of 88.81 who were seen in the different UConn Health clinics between March 31, 2021 and March 31, 2023. The study will be advertised using IRB-approved flyers that will be posted and placed in waiting rooms and clinics (e.g., UConn emergency department, internal medicine, family medicine, geriatric medicine, UConn Urgent Care in Canton, etc.) throughout UConn Health to recruit individuals. Patients will also be identified through daily EPIC reports from the UConn Health outpatient clinics, based on initial screen based on sex, age and ICD10 for metabolic syndrome (E88.81), OR obesity (E66.9) OR overweight (E66) OR a BMI >30. Patient's physician or clinical provider will seek verbal assent from patients for referral to UConn Center on

Aging. Patients will be contacted by our study research coordinator as to their potential interest in participating in the study.

We plan to seek referrals from UConn Health physician and outpatient clinics treating patients with metabolic syndrome and other providers in the community. Potential participants will be notified about the study through their healthcare team (medical doctors, nurses, therapists, social workers, administrative staff, etc.) either by sharing study flyer or thru using a Best Practice Advisory (BPA) referral.

A BPA alert will appear when a UConn Health provider has an encounter with their patients that meet the following criteria, Women, 50 years or older with ICD10 codes for metabolic syndrome, OR obesity (E66.9) OR overweight (E66) OR a BMI>30 . The provider will have the opportunity to ask the patient if they would like to learn more about a study about bladder symptoms being conducted by the UConn Center on Aging. If the patient provides verbal permission to be contacted to the provider, the provider will select "interested" in the BPA alert. If the patient is not interested, the provider will select "declined". If the provider would like to defer the BPA to their storyboard until ready to select a choice, they can do so. The BPA will appear again upon the provider closing the chart. Referred patients will appear in the study coordinator's EPIC in-basket message. The study coordinator will click on the referred patient in the EPIC in-basket to access the patient's medical record to obtain contact information (phone) and referring provider name and contact. Patients who express interest will then be contacted by our study research coordinator as to their potential interest in participating in the study. The BPA will appear for outpatient encounters. We will exclude nursing homes, ED visits and in-patient encounters for this BPA. The BPA will only appear for the following providers: MD, APRN and PA.

With the support of clinical departments at UConn Health and approval from providers in those departments, we will use EPIC to identify potentially eligible patients using the criteria below. Letters will be mailed to these patients with a cover letter on UConn Health letterhead inviting patients to participate in the study; the study's IRB approved flyer will also be enclosed.

- Patient sex is female and sex assigned at birth is female
- Age 50+
- BMI 30+ OR obesity diagnosis
- No prior diagnosis of cancer
- No prior diagnosis of, or current treatment for diabetes
- History of hemoglobin A1C ≥6.5

Recruitment flyers will also be provided to other community healthcare providers to share with patients. Additional recruitment methods will include community talks where participation in the research study will be offered; distribution of flyers to include community housing, churches, organizations, medical clinics, and community centers; and advertising in newspapers/newsletters, posting the study flyer on social media and the internet or on the radio. Educational events will be held at interested senior centers, support groups, or online webinars during which the recruitment flyer will be shared.

Interested subjects will call or email to inquire about the study. Interested subjects will be given a brief explanation of the study and pre-screened via a telephone interview for eligibility. If initial eligibility criteria are met, the subject will be scheduled for a screening visit. We will recruit 50 older women with metabolic syndrome and exhibiting LUTS.

Retention

Enrolled subjects will be given a small monetary payment to recognize their time and effort in study participation. Participant monetary incentives will be paid at screening visit and visit 1, 2, and 3 as outlined below. Participants will receive a total of $250 for the participation in all aspects of the study, screening visit, and 3 study visits. If they miss any visits, the payment will be prorated. In addition to monetary incentives, study retention will be encouraged by establishing a good rapport with the study participant and maintaining a welcoming environment for all study visits. Additionally, regular contact throughout the study will assist in reminding participants of visits and increasing retention throughout the study.

If the participant completes:	**Payment received:**
Screening Visit	$25
Visit 1	$50
Visit 2	$75
Visit 3	$100
Total possible payment:	**$250**

Medication Adherence

To encourage medication adherence, research staff will contact research participants every two weeks (twice weekly for first 3 weeks of enrollment) to check on adherence and any other issues. Additionally, participants will be given a defined amount of medication at visit 1 and will be instructed to bring remaining study medication to visit 2. Medication will be counted to assess for medication adherence. Similarly, a defined amount of medication will be dispensed at visit 2 and participants will be instructed to bring in remaining medication to visit 3. Thus, we will be able to calculate the total medication adherence per participant for the study, e.g. amount of medication taken/amount of medication intended.

If participants have low medication adherence rates or refuse to take medication, the PI will decide if they will be withdrawn from the study at that time. For example, if during the Week 3 Call 2 it is found that a participant is still struggling to or refusing to regularly and properly take the study drug, they will be withdrawn from the study; similarly, PI may wish to withdraw the participant if they have not been taking study medication at the 12 week call. If a subject withdraws or is withdrawn from the interventional portion of the study (i.e, stops taking study drug due to AE or other reason), but is within a 2-week window from a scheduled visit or assessment call, their data will still be collected and included in the study for this following study time point, per PI's discretion.

Protocol Adherence and Complete Data Collection

We will regularly call participants 4-7 days in advance of visits 1, 2 and 3 to remind them about bringing their completed 3-day voiding diary, optional 24-hour urine collection and first morning urine to the visits.
Additionally, to ensure complete data and sample collection, if a participant forgets to fill out or bring their 3-day voiding diary, collect or bring their optional 24-hour urine or first morning urine samples as required for visits 1, 2 and 3, we will ask them to complete these items within the following week (7 days). We will ask participants to bring them into the Center on Aging if they are able to or schedule to have one of our research team members pick it up from their place of residence at an agreed upon time.

RISKS AND PROTECTIONS

Potential Risks to Subjects

There are no major risks associated with participation in this study.

Risk to Confidentiality: There is a potential risk to confidentiality due to the protected health information collected and stored in the subject's research record.
Risk from Blood draw: There may be a minor amount of discomfort due to the phlebotomy. There is a minor risk of bruising (<1%), infection at the phlebotomy site (<1%) or dizziness following the blood draw (<1%). Blood (131ml over study duration) will be drawn peripherally via venipuncture by an experienced, trained research staff in a clinic setting on a hospital campus using best practices. The area where the needle will be inserted will be wiped with alcohol before the draw. A band-aid will be placed over the site. Emergency treatment is accessible on campus for any severe complications from blood draw. Significant hypoglycemia after a normal overnight fast is rare among this population. In order to reduce this risk, phlebotomy will be scheduled early morning, and be provided with breakfast following blood draw. Participants with diabetes requiring insulin for treatment will be excluded from this study.

MitoQ Dietary Supplement and Placebo: MitoQ is an antioxidant vitamin. This study will look to see if lower urinary tract symptoms resulting from aging and metabolic syndrome in older women improve as a result of MitoQ supplementation. MitoQ is not approved by the U.S. Food and Drug Administration (FDA) for the uses being tested in this study. The uses in this study are considered "investigational." However, the FDA has allowed the use of MitoQ in this research study. MitoQ is uniquely designed antioxidant vitamin, used to target cell stress and boost energy.

MitoQ is not a pharmaceutical but rather classified as a dietary supplement. There are no known interactions with medications. Participants will be instructed to take the study drug they are assigned to once a day in the morning with water thirty minutes before breakfast. Participants will be instructed to let the research team know if they experience any side effects or concerns while taking this study drug. This study uses a placebo that will also be supplied by the MitoQ manufacturer for this study.

MitoQ or placebo capsules should be stored in a dry place below 25°C/77°F.

Headaches, nausea, vomiting, mild upset stomach may occur when using the study drug.

There is a possibility that MitoQ may prolong the QT interval and increase the risk of ventricular arrhythmia. Therefore, an ECG assessment to measure the QT interval will be done at screening and Visits 2 and 3 to monitor the QTc after study drug initiation. We will exclude anyone from participating who has a QTc interval on the ECG over 460 ms. If the heart's electrical activity increases over 500 ms or increases more than 50 ms from the participant's baseline measurement after consuming the MitoQ, the study subject will be told to discontinue the study protocol. If the QT interval on the ECG increases over 500 ms or increases more than 50ms from the participant's baseline QT interval measured during the screening visit, participant will undergo a clinical evaluation by the study physician and will be discontinued from the study. The study physician may recommend seeking follow up care with a primary care physician.

Preferably, participants are advised to take the study capsules 30 minutes before breakfast. However, participants may take the supplement with food and water if they have headaches, nausea, and mild upset stomach after using the study drug. To decrease the possibility of gastrointestinal issues, participants will be instructed to take only one capsule per day for the first week of the study and then begin taking two capsules each day. Participants should let the research team know if they experience any side effects or concerns while taking this study drug.

Assessment of Safety

There are no major risks associated with participation in this study. The safety record of MitoQ has been

shown to be excellent up to 80mg daily dose in several human studies. The goal of Mito-LUTS safety management is to minimize the occurrence of adverse events and manage adverse events as they relate to the study. To minimize specific known potential adverse events due to the interventions, Safety Parameters will be assessed as indicated below. The PI, Iman Al-Naggar, will have primary responsibility to ensure the safety of the study participants in relation to the study protocol. She will do this in close collaboration with Dr. George Kuchel, who will be the primary clinical/medical investigator on the study. In consultation with the PI and study staff, Dr. Kuchel will regularly review all health assessments, eligibility criteria, vital signs, medical history, medication history, AE's, ECG and laboratory tests, with a formal comprehensive safety review every 2 months. The Safety Officer (SO), will be unblinded to the intervention and will have the responsibility to monitor study data for evidence of intervention specific adverse events. She will be alerted to any SAEs, UPs, major safety protocol deviations and abnormal laboratory or ECG test results based on recommendation from Dr. Kuchel. The SO will contact the research pharmacist directly to inquire which study arm they are assigned to. The SO, however, will not provide direct medical care to the participants if laboratory tests or health assessments reveal abnormalities, but rather refer them to their primary care physician. The SO will make clinical recommendations regarding the safety of the subjects to continue participating in the study. The SO will review all components every 6 months. Other Study Associated Risks will be minimized as indicated below.

Safety Parameters

Heart Function

A hypothesized side effect of MitoQ is increasing the risk of ventricular arrhythmia. Therefore, an ECG assessment to measure the QT interval will be done at screening and Visits 2 and 3 to monitor the QTc after study drug initiation. We will exclude anyone from participating who has a QTc interval on the ECG over 460 ms. If the QT interval on the ECG is found to have increased over 500 ms or increased more than 50 ms over participant's baseline QT interval at any visit, participants will undergo a clinical evaluation by the study physician and will be discontinued from the study. The study physician may recommend seeking follow up care with a primary care physician. We will use the the Bazett correction for QT interval for heart rates between 60-100 bpm and the Hodges for heart rates above 100 bpm. For slow heart rates, we will use the actual QT since the formulae, especially the Bazett, overcorrect.

Gastrointestinal symptoms

A rare side effect of MitoQ is gastrointestinal symptoms, namely nausea, vomiting and abdominal discomfort. Participants will report on gastrointestinal symptoms at each visit, and during regularly scheduled (twice weekly during first 3 weeks of enrollment and every two weeks afterwards) telephone conversations. If severe gastrointestinal symptoms (vomiting and/or diarrhea) are reported before or during the Week 1 Call 1, and continue for 3 consecutive days, participants will be asked to stop the medication and be withdrawn from the study. If GI symptoms are mild (nausea and abdominal discomfort) at the Week 1 Call 1 but are not bothersome to participants, they will be advised to continue taking the drug; however, if mild GI symptoms persist for 5 consecutive days, they will be asked to stop the study drug and will be withdrawn from the study. If, following the initial drug acclimatization period, participants develop severe GI symptoms for 3 consecutive days, participants will be asked to stop the study drug, the Safety Officer (SO) will be consulted and determine if participants should resume taking the drug or be withdrawn. If new onset (after initial two-week acclimatization period) GI symptoms are mild but persist for 5 days, participants will be asked to stop the study drug, the SO will be consulted and determine if participants should resume taking the drug or be withdrawn.

Kidney function

Participants who have a history of chronic kidney disease or have an eGFR ≤ 60 mL/min/1.73m^2 at screening will be excluded from the study at the study physician's discretion. Current FDA recommends

monitoring for renal function every 3-6 months in those with eGFR between 45-60 mL/min/1.73m^2 and annual monitoring for those with eGFR ≥ 60 mL/min/1.73m^2. Additionally, the FDA recommends more frequent monitoring of renal function in older adults. To that end, eGFR will be evaluated at screening, visit 2 (week 8) and visit 3 (week 16). Those with impaired renal function (eGFR ≤ 60 mL/min/1.73m^2) at screening will be excluded from the study at the study physician's discretion. If glomerular filtration rate is ≤ 50 mL/min/1.73m^2 at visit 2, study medication may be discontinued at the study physician's discretion and participant referred to study physician for evaluation and may be referred to their primary care physician. At the discretion of the PI and safety officer, the participant may be given the option to continue with study visits following cessation of the study medication.

Liver function

Participants with existing hepatic impairment (indicated by 3X the upper limit of normal for ALT and AST or other clinical findings) or a history of alcoholism will be excluded from the study. Liver function will be evaluated at screening, visit 2 and visit 3 (after 8 and 16 weeks of treatment). If ALT/AST are 3X the upper limit of normal at screening, participants will be excluded from the study. If ALT/AST are 3X the upper limit of normal at visit 2, study medication will be discontinued. At the discretion of the PI and safety officer, the participants may be given the option to continue with study visits following cessation of the study medication. Additionally, if enrolled participants have mild to moderate elevated ALT/AST values (1.1 – 2.9X the upper limit of normal) at screening, visit 2 or visit 3, or have worse elevation in ALT/AST levels over time, the participant will be referred to their primary care physician for re-testing and any necessary care.

Other Study Associated Risks

Frailty and Physical Performance Testing

There is a very small risk for injury during the physical function testing. To mitigate these risks, trained staff will conduct all assessments and if participants report chest pain, chest tightness, shortness of breath, lightheadedness, or other issues the testing will be stopped.

Urine Collection

There are no physical risks associated with urine collection.

Questionnaires and Voiding Diary

Participants will be asked about demographics, medical history, current medications, voiding symptoms, voiding parameters, a cognition questionnaire and questionnaires about their study experience. There are no physical risks associated with the questionnaires or voiding diary.

Risk to Confidentiality

We will protect the confidentiality of the participant's data to the best of our ability. The following procedures will be used to protect the confidentiality. Study staff will keep all study records (including any codes to your data) locked in a secure location. All study information will be placed in separate research record and will not be placed in their medical record, except for JDH lab results that will be posted in the medical record. Research records will be labeled with a code and all contents of the research record will be labeled with only that code. A master key that links names and codes will be maintained in a separate and secure location. Any study documents that contain participants' name, such as this informed consent form and HIPAA document, will be kept separate from research records and locked in a secure location.

All electronic files (e.g., database, spreadsheet, etc.) containing identifiable information will be password protected. Any computer hosting such files will also have password protection to prevent access by un-authorized users. Data that will be shared with others will be coded as described above to help protect participant identity. Any laptop computers that will be used will be encrypted. Any data that is shared with other researchers will be coded as described above to protect participants' identify and

will not contain participants' name or any other personal identifiers. All data that is to be shared with the scientific community in online databases will not contain any personal identifiers and will be re-coded to further protect identity.

In order to properly analyze lab results relative to age-appropriate normal value ranges and ensure results obtained correspond to the correct study participant, blood samples sent to John Dempsey Hospital Laboratory for processing will be labeled with participant research medical record number (TO#), name and Date of Birth (DOB). The DOB, TO# and name will then be redacted in printed copies of test results kept in participant binders and replaced with the participant's Study ID. Participants medical record will reflect that they are involved in a research study and JDH Blood Labs will be posted in their medical record.

Results of the study will not identify subjects by name or visit dates. The information collected for this research study will be accessible to authorized persons. Authorized persons include study team members, representatives of UConn Health; representatives of the funding agency (The National Institute on Aging and The Urology Care Foundation and American Urological Association) and representatives from Federal agencies when required by law, such as representatives from the Food and Drug Administration and the Department of Health and Human Services. Representatives from these areas will have access to the information so they may ensure that the study is being done correctly.

Risk Management

Protections Against Risks to Human Subjects

All AEs that are serious, unexpected, and considered related to the study judged by the Investigators will require expedited reporting. All available information relevant to the evaluation of the SAE will be reported.

A Serious Adverse Event (SAE) is any untoward medical occurrence or effect that at any dose:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

Study staff will inquire about potential side effects, AEs, or other incidents during study visits and regular phone calls (twice weekly during first 3 weeks of enrollment and every two weeks after that). Participants will also be instructed to call study staff to report any side effects, medical changes, and AEs throughout the study. Laboratory testing will also be reviewed by research staff or study PI with the study's primary clinical/medical investigator on the study (Dr. Kuchel) or study nurse to determine AEs. All potential AEs will be recorded by staff on the case report form and appropriate source documentation will be collected if possible, e.g. laboratory test reports, hospital charts, or other medical records. The primary clinical/medical investigator on the study (or study nurse) will classify the AE as detailed above. AEs will be reported to the UConn Health IRB and FDA as dictated below.

Unexpected fatal or life-threatening **suspected adverse reactions** will be reported to the UConn Health IRB and FDA as soon as possible, within 7 calendar days following initial receipt of the information as mandated by the FDA.

Unexpected serious **suspected adverse reactions** will be reported to the UConn Health IRB and FDA within 15 calendar days following initial receipt of the information as mandated by the FDA.

Follow-up IND Safety Reports will be submitted to the FDA if any additional information is available

for a reported suspected adverse reactions within 15 calendar days following initial receipt of the information as mandated by the FDA.

Unanticipated problems (AE that is unexpected, serious, and related or possibly study-related) will be reported to the UConn Health IRB within 7 calendar days (5 working days) following initial receipt of the information per UConn Health IRB policy.

All other AEs will be logged and reported to the UConn Health IRB and FDA as mandated.

Action Required During Study Intervention for Adverse Events or Others

Emergency Unblinding

The unblinding of treatment assignment for a subject may be necessary due to a medical emergency or any other significant medical event. Subject safety will always be the first consideration in making such a decision. This may be due to an AE or other incident where it is important to know if the subject is taking MitoQ or placebo. In this case, the PI will consult with the safety officer to confirm unblinding is necessary. If necessary, both the safety officer and the participant will be unblinded to the treatment group. Unblinding will be done by consulting the UConn Pharmacy, which is responsible for randomizing and dispensing study drug. If deemed appropriate by the safety officer and study PI, the participant may remain in the study if they choose to.

Discontinue Medication

The study physician will determine if it is necessary for the participant to discontinue medication based on laboratory testing (outlined above in Safety Assessments), side effects, AEs, or other incidents. If the participant needs to discontinue medication for one of these reasons, they may be asked to continue participating in the study assessments at the discretion of the PI and safety officer.

As outlined above, study medication will be discontinued in participants if laboratory testing (at Visit 2 after approximately 8 weeks of treatment) reveals any of the following:

- Renal impairment indicated by an eGFR $\leq$ 50 mL/min/1.73m^2
- Hepatic impairment indicated by ALT/AST levels that are 3X the upper limit of normal
- QTc interval on ECG of 500ms or higher or QTc interval increased 50ms or more from baseline measured at screening visit.

The participants will also be advised to discontinue study medication in the following situations:

- Participants will be instructed to discontinue study medication prior to iodinated contrast imaging procedures due to acute alterations in kidney function. Treatment will be restarted if re-evaluation of eGFR at least 48 hours after the procedure is within target range ($\geq$50 mL/min/1.73m^2)

Participants will be requested to discontinue study medication if:

- They experience severe GI discomfort (vomiting and/or diarrhea) that does not resolve within 3 days.
- They experience mild GI discomfort (nausea or abdominal discomfort) that does not resolve within 5 days.

Study-Stopping Criteria:

The study will be discontinued if any of the following is observed in 10 participants (i.e., 20% of planned study size):

- Renal impairment indicated by an eGFR $\leq$ 50 mL/min/1.73m^2
- Hepatic impairment indicated by ALT/AST levels that are 3X the upper limit of normal.
- Increased QT interval on ECG > 500 ms or by 50 ms from baseline.

Data and Statistical Considerations

Tests and Assays

Some spot urine will be immediately used for urinalysis by dipstick. Serum, plasma and urine will be collected and stored at -80C for future analyses. Biomarkers of aging, oxidative stress, inflammation, senescence, and mitochondrial function will be measured in plasma, serum and urine. Measurements will be done at the UConn Pepper Center Biomarkers Core (RC3) and the Facility for Geroscience Analysis at Mayo Clinic.

Sample size consideration

Proposed sample sizes for pilot and feasibility range from 24, to 30 and 50 [51-53]. As sample sizes between 24 and 50 have been recommended for pilot and feasibility studies to help determine SD in parameters (such as the Lower urinary tract symptoms questionnaire scores we will be assessing as outcome measures in this proposed study), we will use a sample size of 50. However, although as mentioned above a formal sample size calculation is not appropriate for the type of study proposed, we provide an example such calculation for Aim 1. Aim 1: Estimates of standard deviations (SD) for Overactive Bladder Symptom Score (OABSS) come from Chuang et al [54] who report SD in the range of 1.1 to 2.2 for patients with LUTS similar to our study. It is generally accepted that mean improvements in OABSS of 3 are clinically meaningful[55,56]. A sample size of 20 in each group will have 99% power to detect a difference in means of 3 assuming SD=2.2 using a two group t-test with a 0.05 two-sided significance level. Our planned analysis of covariance (ANCOVA) models should have even greater power characteristics than *t*-tests. Aim 2: We consider Aim 2 to be exploratory and supportive to Aim 1. No sample size calculations were done as the sample size was established at 50 based on recommendations for pilot and feasibility studies.

Block Randomization

We will use stratified block randomization for the following four groups so that age and BMI are as balanced as possible for this study:

Group 1: Age 50-64 and BMI < 35

Group 2: Age 50-64 and BMI ≥ 35

Group 3: Age ≥65and BMI < 35

Group 4: Age ≥65and BMI ≥ 35

For each of the above groups, we will use blocks of size 3 and size 6 (one block of size 6 with others size 3) to achieve a target sample size of 4 women into the placebo group and 8 women into the MitoQ treatment group (i.e., a 1:2 ratio). Randomization sequence for treatment allocation will be changed for each block by using a permuted block randomization approach to help prevent study staff from being able to guess treatment assignments and remain blinded. Our ultimate goal is to have well-balanced placebo and study drug groups in terms of age and BMI distribution.

Randomization lists will be provided by a statistician.

Statistical Analyses

We will primarily use analysis of covariance (ANCOVA) to compare mean scores at week 16 between the placebo and MitoQ-treated group. Baseline values will be included as covariates. As the data set is small, perhaps one covariate such as age can be included. Repeated measures general linear models will be considered if the linear trends suggest an analysis with all timepoints is informative. Descriptive analyses will include paired *t*-tests for comparing LUTS questionnaires from Visits 2 and 3 with baseline (Visit 1), separately by group. Voiding diary measures (eg, number of trips to the bathroom during the day or at night, number of episodes of leakiness, number of urgency episodes) will be secondary outcomes and will be compared as done for questionnaire scores. We anticipate that the MitoQ-treated group will have a significant reduction in their LUTS severity (based on both

questionnaire scores and voiding diary data) from baseline and compared to Placebo.

Ethics and Protection of Human Subjects

Ethical Standard

Mito-LUTS will be conducted under full conformity with the Regulations for the Protection of Human Subjects of Research and ICH E6 Good Clinical Practice.

Institutional Review Board

Mito-LUTS will use the University of Connecticut Health IRB. IRB Approval for the protocol, informed consent form, recruitment materials, and source documents will be obtained before any study procedures begin.

Privacy of Subjects

To ensure privacy of study participants, all visits, study procedures and consent are performed in private rooms and areas in the Center on Aging Clinical Research Area.

Consent Process

Women aged 50 years or older with metabolic syndrome or suspected of having the metabolic syndrome per IDF 2006 guidelines will be invited to learn more about this study through study advertisement, and physician referral. Interested subjects will be given a brief explanation of the study and pre-screened via a telephone interview for eligibility by the study research team. If initial eligibility criteria are met, the subject will be scheduled for a screening visit. At the beginning of the screening visit, qualified study staff will lead the potential participant through the consent process in a quiet and private research room at the Center on Aging Clinical Research area. The IRB approved ICF will be thoroughly reviewed with the potential participant and study staff will provide answers to any questions the potential participants may have. Extensive discussion of the possible risks and benefits will be provided to the participant. The participants will be given time to think about the study and discuss with family or others if they feel necessary. If the participant understands and agrees to all aspects of the informed consent form, they will sign the ICF. We will provide a signed copy of the ICF to the subject to keep for their records. It will be emphasized that consent can be withdrawn at any time throughout the study and that the quality of their medical care will not be adversely affected if they choose not to participate.

Confidentiality of Data

Subjects will be assigned a unique research identification number and all samples and data generated will be identified via their unique identifier and time point of collection if applicable, not any personal identifiers. Safeguards to ensure the security and privacy of the participants' records will be taken. The files matching participants' names and demographic information with research ID numbers will be kept in a separate locked filing cabinet and/or in a separate password-protected database on a secure network drive to which only authorized personnel have access. Hard copies of the research records (source documents from each visit) will be kept in a locked filing cabinet and will be identified by research ID number only. Electronic research records will be stored in password-protected databases on a secure network drive. Only approved study personnel have access to these databases and passwords.

Biological samples will be identified by research ID number only and no personal identifiers. The biological samples will be used in the experiments outlined and potentially others in the future. Biological samples may be used by investigators other than those listed here in the future if the participant consents to this. Any shared biological samples or data will be identified by research ID number only with no personal identifiers maintaining the confidentiality of the participants.

Data and Safety Monitoring

The Data & Safety Monitoring Plan for this study describes the components of the study that will be monitored by the PI, study coordinators and data manager. Recruitment, dropouts, adverse events, unanticipated problems, data integrity and confidentiality, participant privacy and the general conduct of the study will be reviewed throughout the study as they arise and at team meetings, unless it is determined

to be addressed sooner. Summaries of DSMP review(s) will be kept in the regulatory binder and provided to IRB at study continuation. Study samples are limited to urine and peripheral blood draws collected from study participants.

Procedures in place to ensure confidentiality of research data are as follows:

1. Only authorized individuals have access to any data, used or stored (via electronic format or as hard copy records). Only designated research staff and principal investigators will be granted access.

2. Logon IDs and passwords for access to the UConn Center on Aging shared network drive and the University's online resources are initially assigned by the Information Technology Department (IT).

3. All data (clinical, recruitment and schedule-based) are stored in password-protected databases on the secured network drive. Only approved personnel have access to these databases and passwords.

All data collected on data forms are stored in locked drawers located in the Center on Aging research facility. Files are stored in locked cabinets in rooms that are locked when not in use. All data are backed up on the shared network drive for UCHC.

Data Management

Dr. Al-Naggar will assume primary responsibility for data accuracy and protocol compliance. Data will be collected securely and double-checked by research staff. Audits of randomly selected participant files will be conducted monthly to check on accuracy of data entry and protocol adherence. Adherence to protocols will also be ensured by ongoing supervision by Dr. Al-Naggar, who will report regularly to Drs. Kuchel and Albertsen in their regularly scheduled meetings. Protocol deviations as a result of staff error will be reported to the IRB with a protocol deviation report form. The protocol deviation report will outline the root cause of the problem and establish a plan of action to avoid similar errors in the future. Minor protocol deviations will be reported annually to the IRB as per the regulatory guidelines. Data will be encrypted, password protected, and stored on UConn Health IT servers. Access to clinical research records and identifiable study data will be restricted to the study team involved in the conduct of the clinical protocol.

Informed Consents and HIPAA documents are stored in a separate file apart from participant's research records.

Protocol Deviations

A cumulative Protocol Deviation Log will be kept electronically by the study coordinators with a copy added to the regulatory binder. Deviations from the protocol will be entered on this log with a Note to File labeled with PID and no personal identifiers added to the regulatory binder with a copy to the research record that describes the deviation, date when it was identified, the corrective action taken to prevent recurrence, whether or not the deviation met criteria of an Unanticipated Problem, and the date of IRB notification.

Incidents of non-compliance, defined as any action that is taken or occurs that is not in accordance with an IRB approved study, IRB policies or regulations or failure to follow the requirements and determinations of the IRB, that is within the control of the study team must be reported to the IRB within 5 days of PI becoming aware of the occurrence.

Unanticipated Problems

For the purpose of reporting Unanticipated Problems to IRB at all sites, internal adverse events that may also represent an unanticipated problem are defined as those events, experiences or outcomes that are:

1. Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are

described in the protocol-related documents, such as the IRB approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; and

2. Related or possibly related to participation in the research (i.e., there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research). Any internal event meeting these criteria must be reported to the IRB, which will then make the final determination as to whether the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social) than was previously recognized.

Budget/Resources

This study will be funded by the National Institutes of Health / National Institute on Aging's Claude D. Pepper Older Americans Independence Center & UConn Health (P30 AG067988) **and** The Urology Care Foundation and American Urological Association New England Section Wyland F. Leadbetter, MD Award (Research Scholar Award) awards to Dr. Iman Al-Naggar (PI).

Dissemination

Results from this pilot study will be used for obtaining NIH funding through R-award applications, presented at conferences and published in peer-reviewed journals such as Geroscience, Aging Cell and Nature Medicine. Results of this study when published or presented will not identify subjects by name.

References

1. Vaughan CP, Markland AD, Smith PP, et al. Report and Research Agenda of the American Geriatrics Society and National Institute on Aging Bedside-to-Bench Conference on Urinary Incontinence in Older Adults: A Translational Research Agenda for a Complex Geriatric Syndrome. *J Am Geriatr Soc* 2018; **66**(4): 773-82.
2. Rubin EB, Buehler AE, Halpern SD. States Worse Than Death Among Hospitalized Patients With Serious Illnesses. *JAMA Intern Med* 2016; **176**(10): 1557-9.
3. Moga DC, Abner EL, Wu Q, Jicha GA. Bladder antimuscarinics and cognitive decline in elderly patients. *Alzheimers Dement (N Y)* 2017; **3**(1): 139-48.
4. Araklitis G, Robinson D, Cardozo L. Cognitive Effects of Anticholinergic Load in Women with Overactive Bladder. *Clin Interv Aging* 2020; **15**: 1493-503.
5. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013; **153**(6): 1194-217.
6. Schmauck-Medina T, Moliere A, Lautrup S, et al. New hallmarks of ageing: a 2022 Copenhagen ageing meeting summary. *Aging (Albany NY)* 2022; **14**(16): 6829-39.
7. Sierra F, Kohanski R. Geroscience and the trans-NIH Geroscience Interest Group, GSIG. *Geroscience* 2017; **39**(1): 1-5.
8. Chaib S, Tchkonia T, Kirkland JL. Cellular senescence and senolytics: the path to the clinic. *Nat Med* 2022; **28**(8): 1556-68.
9. Inouye SK, Studenski S, Tinetti ME, Kuchel GA. Geriatric syndromes: clinical, research, and policy implications of a core geriatric concept. *J Am Geriatr Soc* 2007; **55**(5): 780-91.
10. Bunn F, Kirby M, Pinkney E, et al. Is there a link between overactive bladder and the metabolic syndrome in women? A systematic review of observational studies. *Int J Clin Pract* 2015; **69**(2): 199-217.
11. Sebastianelli A, Gacci M. Current Status of the Relationship Between Metabolic Syndrome and Lower Urinary Tract Symptoms. *Eur Urol Focus* 2018; **4**(1): 25-7.
12. De Nunzio C, Cindolo L, Gacci M, et al. Metabolic syndrome and lower urinary tract symptoms in patients with benign prostatic enlargement: a possible link to storage symptoms. *Urology* 2014; **84**(5): 1181-7.

13. Hsu LN, Hu JC, Chen PY, Lee WC, Chuang YC. Metabolic Syndrome and Overactive Bladder Syndrome May Share Common Pathophysiologies. *Biomedicines* 2022; **10**(8).
14. Ponholzer A, Temml C, Wehrberger C, Marszalek M, Madersbacher S. The association between vascular risk factors and lower urinary tract symptoms in both sexes. *Eur Urol* 2006; **50**(3): 581-6.
15. Kirkland JL. Inflammation and Cellular Senescence: Potential Contribution to Chronic Diseases and Disabilities With Aging. *Public Policy & Aging Report* 2013; **23**(4): 12-5.
16. Kupelian V, Rosen RC, Roehrborn CG, Tyagi P, Chancellor MB, McKinlay JB. Association of overactive bladder and C-reactive protein levels. Results from the Boston Area Community Health (BACH) Survey. *BJU Int* 2012; **110**(3): 401-7.
17. Tyagi P, Tyagi V, Qu X, et al. Association of inflammaging (inflammation + aging) with higher prevalence of OAB in elderly population. *Int Urol Nephrol* 2014; **46**(5): 871-7.
18. Tyagi P, Barclay D, Zamora R, et al. Urine cytokines suggest an inflammatory response in the overactive bladder: a pilot study. *Int Urol Nephrol* 2010; **42**(3): 629-35.
19. Siddiqui NY, Helfand BT, Andreev VP, et al. Biomarkers Implicated in Lower Urinary Tract Symptoms: Systematic Review and Pathway Analyses. *J Urol* 2019; **202**(5): 880-9.
20. Helfand BT, Andreev VP, Siddiqui NY, et al. A Novel Proteomics Approach to Identify Serum and Urinary Biomarkers and Pathways that Associate with Lower Urinary Tract Symptoms in Men and Women: Pilot Results of the Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN) Study. *Urology* 2019; **129**: 35-42.
21. Chen L, He PL, Yang J, et al. NLRP3/IL1beta inflammasome associated with the aging bladder triggers bladder dysfunction in female rats. *Mol Med Rep* 2019; **19**(4): 2960-8.
22. Inouye BM, Hughes FM, Jr., Sexton SJ, Purves JT. The Emerging Role of Inflammasomes as Central Mediators in Inflammatory Bladder Pathology. *Curr Urol* 2018; **11**(2): 57-72.
23. Andersson KE. Oxidative stress and its possible relation to lower urinary tract functional pathology. *BJU Int* 2018; **121**(4): 527-33.
24. Song QX, Sun Y, Deng K, Mei JY, Chermansky CJ, Damaser MS. Potential role of oxidative stress in the pathogenesis of diabetic bladder dysfunction. *Nat Rev Urol* 2022; **19**(10): 581-96.
25. Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders - A step towards mitochondria based therapeutic strategies. *Biochim Biophys Acta Mol Basis Dis* 2017; **1863**(5): 1066-77.
26. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *Int J Mol Sci* 2019; **20**(13).
27. Azadzoi KM, Siroky MB. Mechanisms of Lower Urinary Tract Symptoms in Pelvic Ischemia. *J Biochem Pharmacol Res* 2013; **1**(1): 64-74.
28. Yang JH, Choi HP, Niu W, Azadzoi KM. Cellular Stress and Molecular Responses in Bladder Ischemia. *Int J Mol Sci* 2021; **22**(21).
29. Lin WY, Andersson KE, Lin CL, Kao CH, Wu HC. Association of lower urinary tract syndrome with peripheral arterial occlusive disease. *PLoS One* 2017; **12**(3): e0170288.
30. Pinggera GM, Mitterberger M, Steiner E, et al. Association of lower urinary tract symptoms and chronic ischaemia of the lower urinary tract in elderly women and men: assessment using colour Doppler ultrasonography. *BJU Int* 2008; **102**(4): 470-4.
31. Luft R, Landau BR. Mitochondrial medicine. *J Intern Med* 1995; **238**(5): 405-21.
32. Sorescu D, Griendling KK. Reactive oxygen species, mitochondria, and NAD(P)H oxidases in the development and progression of heart failure. *Congest Heart Fail* 2002; **8**(3): 132-40.
33. de Rijk MM, Wolf-Johnston A, Kullmann AF, et al. Aging-Associated Changes in Oxidative Stress Negatively Impacts the Urinary Bladder Urothelium. *Int Neurourol J* 2022; **26**(2): 111-8.
34. Birder LA, Wolf-Johnston A, Wein AJ, et al. Purine nucleoside phosphorylase inhibition ameliorates age-associated lower urinary tract dysfunctions. *JCI Insight* 2020; **5**(20).
35. Kim JW, Jang HA, Bae JH, Lee JG. Effects of coenzyme Q10 on bladder dysfunction induced by

chronic bladder ischemia in a rat model. *J Urol* 2013; **189**(6): 2371-6.
36. Mossa AH, Shamout S, Cammisotto P, Campeau L. Urinary metabolomics predict the severity of overactive bladder syndrome in an aging female population. *Int Urogynecol J* 2020; **31**(5): 1023-31.
37. Nomiya M, Andersson KE, Yamaguchi O. Chronic bladder ischemia and oxidative stress: new pharmacotherapeutic targets for lower urinary tract symptoms. *Int J Urol* 2015; **22**(1): 40-6.
38. Smith RA, Murphy MP. Animal and human studies with the mitochondria-targeted antioxidant MitoQ. *Ann N Y Acad Sci* 2010; **1201**: 96-103.
39. Murphy MP, Smith RA. Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annu Rev Pharmacol Toxicol* 2007; **47**: 629-56.
40. Dashdorj A, Jyothi KR, Lim S, et al. Mitochondria-targeted antioxidant MitoQ ameliorates experimental mouse colitis by suppressing NLRP3 inflammasome-mediated inflammatory cytokines. *BMC Med* 2013; **11**: 178.
41. Han Y, Xu X, Tang C, et al. Reactive oxygen species promote tubular injury in diabetic nephropathy: The role of the mitochondrial ros-txnip-nlrp3 biological axis. *Redox Biol* 2018; **16**: 32-46.
42. Chen S, Wang Y, Zhang H, et al. The Antioxidant MitoQ Protects Against CSE-Induced Endothelial Barrier Injury and Inflammation by Inhibiting ROS and Autophagy in Human Umbilical Vein Endothelial Cells. *Int J Biol Sci* 2019; **15**(7): 1440-51.
43. Dou SD, Zhang JN, Xie XL, et al. MitoQ inhibits hepatic stellate cell activation and liver fibrosis by enhancing PINK1/parkin-mediated mitophagy. *Open Med (Wars)* 2021; **16**(1): 1718-27.
44. Xiao L, Xu X, Zhang F, et al. The mitochondria-targeted antioxidant MitoQ ameliorated tubular injury mediated by mitophagy in diabetic kidney disease via Nrf2/PINK1. *Redox Biol* 2017; **11**: 297-311.
45. Rossman MJ, Santos-Parker JR, Steward CAC, et al. Chronic Supplementation With a Mitochondrial Antioxidant (MitoQ) Improves Vascular Function in Healthy Older Adults. *Hypertension* 2018; **71**(6): 1056-63.
46. Gioscia-Ryan RA, LaRocca TJ, Sindler AL, Zigler MC, Murphy MP, Seals DR. Mitochondria-targeted antioxidant (MitoQ) ameliorates age-related arterial endothelial dysfunction in mice. *J Physiol* 2014; **592**(12): 2549-61.
47. Gioscia-Ryan RA, Battson ML, Cuevas LM, Eng JS, Murphy MP, Seals DR. Mitochondria-targeted antioxidant therapy with MitoQ ameliorates aortic stiffening in old mice. *Journal of Applied Physiology* 2018; **124**(5): 1194-202.
48. Park S-Y, Kwon OS, Andtbacka RHI, et al. Age-related endothelial dysfunction in human skeletal muscle feed arteries: the role of free radicals derived from mitochondria in the vasculature. *Acta Physiologica* 2018; **222**(1): e12893.
49. Liu HT, Chancellor MB, Kuo HC. Decrease of urinary nerve growth factor levels after antimuscarinic therapy in patients with overactive bladder. *BJU Int* 2009; **103**(12): 1668-72.
50. Billingham SA, Whitehead AL, Julious SA. An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United Kingdom Clinical Research Network database. *BMC Med Res Methodol* 2013; **13**: 104.
51. Sim J, Lewis M. The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency. *J Clin Epidemiol* 2012; **65**(3): 301-8.
52. Lancaster GA, Dodd S, Williamson PR. Design and analysis of pilot studies: recommendations for good practice. *J Eval Clin Pract* 2004; **10**(2): 307-12.
53. Browne RH. On the use of a pilot sample for sample size determination. *Stat Med* 1995; **14**(17): 1933-40.
54. Chuang FC, Hsiao SM, Kuo HC. The Overactive Bladder Symptom Score, International Prostate Symptom Score-Storage Subscore, and Urgency Severity Score in Patients With Overactive Bladder and Hypersensitive Bladder: Which Scoring System is Best? *Int Neurourol J* 2018; **22**(2): 99-106.
55. Gotoh M, Homma Y, Yokoyama O, Nishizawa O. Responsiveness and minimal clinically

important change in overactive bladder symptom score. *Urology* 2011; **78**(4): 768-73.
56. Kinjo M, Masuda K, Nakamura Y, Taguchi S, Tambo M, Fukuhara H. Does metabolic syndrome influence the efficacy of mirabegron treatment in female patients with overactive bladder? *Int Urogynecol J* 2022.
57. Golden SH, Robinson KA, Saldanha I, Anton B, Ladenson PW. Clinical review: Prevalence and incidence of endocrine and metabolic disorders in the United States: a comprehensive review. *J Clin Endocrinol Metab* 2009; **94**(6): 1853-78.
58. Snow BJ, Rolfe FL, Lockhart MM, et al. A double-blind, placebo-controlled study to assess the mitochondria-targeted antioxidant MitoQ as a disease-modifying therapy in Parkinson's disease. *Mov Disord* 2010; **25**(11): 1670-4.
59. Park SY, Pekas EJ, Headid RJ, 3rd, et al. Acute mitochondrial antioxidant intake improves endothelial function, antioxidant enzyme activity, and exercise tolerance in patients with peripheral artery disease. *Am J Physiol Heart Circ Physiol* 2020; **319**(2): H456-H67.
60. Cardozo L, Coyne KS, Versi E. Validation of the urgency perception scale. *BJU Int* 2005; **95**(4): 591-6.
61. Homma Y, Yoshida M, Seki N, et al. Symptom assessment tool for overactive bladder syndrome--overactive bladder symptom score. *Urology* 2006; **68**(2): 318-23.
62. Abrams P, Avery K, Gardener N, Donovan J, Board IA. The International Consultation on Incontinence Modular Questionnaire: www.iciq.net. *J Urol* 2006; **175**(3 Pt 1): 1063-6; discussion 6.
63. Yamaguchi O, Nishizawa O, Takeda M, et al. Clinical guidelines for overactive bladder. *Int J Urol* 2009; **16**(2): 126-42.
64. Cella D, Smith AR, Griffith JW, et al. A new outcome measure for LUTS: Symptoms of Lower Urinary Tract Dysfunction Research Network Symptom Index-29 (LURN SI-29) questionnaire. *Neurourol Urodyn* 2019; **38**(6): 1751-9.
65. Coyne KS, Matza LS, Thompson C, Jumadilova Z, Bavendam T. The responsiveness of the OAB-q among OAB patient subgroups. *Neurourol Urodyn* 2007; **26**(2): 196-203.
66. Coyne KS, Matza LS, Thompson CL. The responsiveness of the Overactive Bladder Questionnaire (OAB-q). *Qual Life Res* 2005; **14**(3): 849-55.
67. Matza LS, Thompson CL, Krasnow J, Brewster-Jordan J, Zyczynski T, Coyne KS. Test-retest reliability of four questionnaires for patients with overactive bladder: the overactive bladder questionnaire (OAB-q), patient perception of bladder condition (PPBC), urgency questionnaire (UQ), and the primary OAB symptom questionnaire (POSQ). *Neurourol Urodyn* 2005; **24**(3): 215-25.
68. Dyer KY, Xu Y, Brubaker L, et al. Minimum important difference for validated instruments in women with urge incontinence. *Neurourol Urodyn* 2011; **30**(7): 1319-24.
69. Blaivas JG, Panagopoulos G, Weiss JP, Somaroo C, Chaikin DC. The urgency perception score: validation and test-retest. *J Urol* 2007; **177**(1): 199-202.
70. Welsh KA, Breitner, John C. S., Magruder-Habib, Kathryn M. . Detection of Dementia in the Elderly Using Telephone Screening of Cognitive Status. *Neuropsychiatry, Neuropsychology & Behavioral Neurology* 1993; **6**(2): 103-10.
71. Cook SE, Marsiske M, McCoy KJ. The use of the Modified Telephone Interview for Cognitive Status (TICS-M) in the detection of amnestic mild cognitive impairment. *J Geriatr Psychiatry Neurol* 2009; **22**(2): 103-9.
72. Knopman DS, Roberts RO, Geda YE, et al. Validation of the telephone interview for cognitive status-modified in subjects with normal cognition, mild cognitive impairment, or dementia. *Neuroepidemiology* 2010; **34**(1): 34-42.
73. Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. *Lancet* 2005; **366**(9491): 1059-62.
74. Tan CE, Ma S, Wai D, Chew SK, Tai ES. Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? *Diabetes Care* 2004;

27(5): 1182-6.
75. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006; **23**(5): 469-80.
76. Peer N, Steyn K, Levitt N. Differential obesity indices identify the metabolic syndrome in Black men and women in Cape Town: the CRIBSA study. *J Public Health (Oxf)* 2016; **38**(1): 175-82.
77. Aschner P, Buendia R, Brajkovich I, et al. Determination of the cutoff point for waist circumference that establishes the presence of abdominal obesity in Latin American men and women. *Diabetes Res Clin Pract* 2011; **93**(2): 243-7.
78. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ* 2005; **173**(5): 489-95.
79. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001; **56**(3): M146-56.
80. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. *Lancet* 2013; **381**(9868): 752-62.