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ENabling Reduction of low-Grade Inflammation in Seniors (ENRGISE) Pilot study: Concept, rationale and design

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Abstract

Background—Low-grade chronic inflammation, characterized by elevations in Interleukin-6 (IL-6), is an independent risk factor for impaired mobility and slow walking speed in older adults.

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Author Contributions: All authors: study concept, critical revisions of content and approval of final version for publication; TMM: lead the initial draft, revisions of the manuscript, the study design, and selection of outcomes; SDA: contributed to the initial manuscript draft, revisions and review; DPB: provided critical review and revisions of the manuscript; JAC: provided critical review and revisions of the manuscript; MAE: provided critical review and revisions of the manuscript; RAF: contributed to the study design, selection of outcomes, and provided critical review of manuscript; CL: provided critical review and revisions of the manuscript; KHL: provided critical review of the manuscript; CKL: provided critical review and revisions of the manuscript; MMM: provided critical review of the manuscript; MEM: provided critical review and revisions of the manuscript; RPT: provided critical review and revisions of the manuscript; JW: provided critical review and revisions of the manuscript; BR: provided critical review and revisions of the manuscript; JDL: provided critical review and revisions of the manuscript; BR: provided critical review and revisions of the manuscript; JL: contributed to the project organization, development of tables and facilitated manuscript development; CS: contributed to the study outcomes and operational procedures; SW: contributed to the statistical design, provided critical review and revisions of the manuscript; ABN: provided critical review and revisions of the manuscript; WTA: Co-Principal Investigator, secured funding and provided critical review and revisions of the manuscript; MP: Administrative Principal Investigator, responsible for the study concept, secured funding, and provided critical review and revisions of the manuscript.

Design—The ENRGISE (ENabling Reduction of low-Grade Inflammation in SEniors) Pilot study was a multicenter, double-blinded, placebo-controlled randomized pilot trial of two interventions to reduce IL-6 levels.

Setting—Five university-based research centers.

Participants—The target enrollment was 300 men and women aged ≥70 years who have an average plasma IL-6 >2.5 and <30 pg/ml across 2 measures, separated by at least one week. Participants had low to moderate physical function, defined as 1) self-reported difficulty walking ¼ mile or climbing a flight of stairs and 2) a usual walk speed <1 m/sec on a 4 m usual paced walk.

Intervention—Participants were randomized to Losartan (LO), omega-3 (ω-3) fish oil, combined LO+ω-3 or placebo. Randomization was stratified depending on eligibility for each group. A titration schedule was implemented to reach a dose that was safe and effective on IL-6 reduction. Maximal doses were 100 mg/day for LO and 2.8 g/day for ω-3 fish oil.

Measurements—IL-6, walking speed over 400 meters, physical function (Short Physical Performance Battery), other inflammatory markers, safety, tolerability, frailty domains, and maximal leg strength were measured.

Results—The ENRGISE Pilot Study was designed to determine recruitment yields, feasibility, medication tolerance and adherence, and to provide preliminary data to help justify a sample size for a more definitive randomized trial.

Conclusions—The ENRGISE Pilot Study will inform a larger subsequent trial that is expected to have important clinical and public health implications for the growing population of older adults with low-grade chronic inflammation and mobility limitations.

INTRODUCTION

The immune system has been actively studied in the aging process since the seminal work by Walford in 1969.[1] Subsequently, a large body of evidence has accumulated demonstrating a chronic, low-grade elevation of inflammatory markers with increasing age.[2] These markers appear to derive in part from senescent cells, which are characterized by growth arrest in response to accumulated damage. Senescent cells secrete cytokines such as IL-6, chemokines, and proteases and are resistant to apoptosis. While these senescent cells are less prone to malignant transformation, they may disrupt normal tissue function, promoting central adiposity, atherosclerotic plaque, and osteoporosis.[3, 4] Moreover, age-related renal dysfunction[5] and atherosclerosis[6, 7] may contribute to the elevation of inflammatory markers through decreased excretion and increased production of these markers. Thus, “chronic inflammation” of aging may indicate an ongoing process of age-related damage and repair resulting a cumulative burden of disease-related damage, and cellular senescence.

While conditions such as myocardial infarction, stroke, hip fracture, and arthritis contribute to disability and inflammation, growing evidence suggests that low-grade chronic inflammation, characterized by elevations in plasma C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), and particularly interleukin-6 (IL-6),[8–11] can directly impair muscle function and are independent risk factors for disability, impaired mobility, and slow

walking speed.[12, 13] Based on current knowledge about the detrimental effects of chronic inflammation, it seems logical that blocking the effects or reducing the sources of chronic low-grade inflammation would have clinical benefit. Alternatively, inflammatory pathways are tightly regulated so that manipulation could have adverse effects such as increased risk of infection or delayed healing. Therefore, it's unclear whether the observed increases in inflammation with age are detrimental and/or adaptive. A randomized trial targeting inflammatory pathways directly was warranted to decide between these alternative interpretations.

The ENRGISE (ENabling Reduction of low-Grade Inflammation in SEniors) Pilot study was a randomized clinical trial (RCT) to gather preliminary data to test whether anti-inflammatory interventions improve or preserve walking ability. Older persons with mobility impairment and elevated levels of inflammation were enrolled— a population at high risk of major mobility disability.[8, 9, 14] The aims of the ENRGISE Pilot study were to compare with placebo, the effects of Losartan (LO), omega-3 fish oil (ω-3) also known as n-3 long chain polyunsaturated fatty acids, and LO+ω-3 on IL-6 and walking speed in 300 older adults (70+ years of age) over 12-months of follow-up. Secondary aims were to evaluate recruitment yields, eligibility criteria, adherence, retention, tolerability, sample-size, design, and parameters affecting the cost for the main RCT focused on major mobility disability as an outcome. The study also aimed to examine intra-subject variability of IL-6, dosage, safety, and other established and novel inflammatory markers. These aims were designed to inform the feasibility of the full ENRGISE trial to assess whether targeting inflammation reduces the risk of major mobility disability. This paper provides the background, rational, conceptual basis, discussion of treatment choices, and research design of the ENRGISE-Pilot Study.

METHODS

Participants and eligibility criteria

Men and women aged 70+ years (n=300) who self-reported difficulty walking ¼ mile or climbing a flight of stairs, had a usual walking speed <1 m/sec on the 4 m walk, and had evidence of chronic low-grade inflammation (IL-6 values >2.5 pg/ml and <30 pg/ml) were enrolled. The value of IL-6 >2.5 pg/ml was chosen because of its association with risk of mobility limitation[15, 16] and persons with ≥30 pg/ml were excluded because they were likely to have acute infections (urinary, respiratory or other) that did not coincide with chronic low-grade inflammation. To reduce the impact of within person variability, IL-6 levels were based on the average of 2 measures taken 1–3 weeks apart, with the first measure being >2.3 and <30 pg/ml, and the average of the two measures >2.5 and <30 pg/ml. The lower value of >2.3 pg/ml at the first measure was designed to be more inclusive of individuals with chronic low-grade inflammation accounting for day-to-day variability and inter-assay variability. Complete eligibility criteria are listed in Table 1.

Participants were excluded if they reported acute infection, autoimmune disease, severe arthritis, and neurologic conditions causing low walking speed. Initially, participants were also excluded for having low vitamin D levels as required by Funding Opportunity Announcement. This requirement was dropped a third of the way through the recruitment

period because of a lack of evidence for how low vitamin D levels would impact the association between inflammation and physical function. Those who took an angiotensin converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB) or potassium sparing diuretics, were excluded from LO randomization. Those who ate >2 servings/week of fish in the past year or taking fish oil were excluded from ω-3 randomization. Recruitment targets were approximately 69% women, 20% racial minorities, and 5% Hispanic or Latino minorities, that reflected the population distribution of these subgroups in the catchment areas. The institutional review boards at each study site approved the protocol and all participants provided written informed consent. The trial is registered as clinicaltrials.gov identifier: NCT01072500.

Study measures and outcomes

A timetable of measures and assessments is listed in Table 2. IL-6 and walking speed during the 400 m walk test were identified as co-primary outcomes of the ENRGISE Pilot study. The 400 m walk test at usual pace was used to assess major mobility disability (MMD),[17] defined as inability to walk ¼ mile. MMD was operationalized as the inability to complete a 400 m walk test within 15 min without sitting or help of another person or walker. In cases when the 400 m walk was not attempted, MMD was adjudicated based on objective inability to walk 4 m in <10 sec, self- or proxy- reported inability to walk across a room or medical record documentation of mobility status.[17]

IL-6 was prioritized over other inflammatory factors because it is a stable inflammatory marker that is less sensitive to day-to-day and diurnal variations and the marker most consistently associated with mobility limitations.[15, 16] IL-6 levels were determined using a sandwich immunoassay (Human IL-6 Quantikine ELISA Kit, R & D Sytems, Minneapolis, MN, catalog #HS600B). Levels greater that 10 pg/ml were evaluated with a different assay with greater dynamic range (Human IL-6 QuantiGlo ELISA Kit, R & D Systems, catalog #Q6000B). The collection, preparation, handling and storage of biological samples followed standard operating procedure that complied with Office of Extramural Research requirements and guidelines.

Secondary outcomes included physical performance, frailty, muscle strength and inflammatory biomarkers to characterize the effect of the interventions.

- Physical performance was measured on the Short Physical Performance Battery (SPPB), which is based on a timed 4 m walk, balance & chair stands tests.[18]
- Frailty was characterized using the Fried criteria that employed self-reported exhaustion, unintentional weight loss, low energy expenditure, slow gait speed, and weak grip strength.[19]
- Muscle strength was measured with maximal tests of isometric grip strength and isokinetic leg extension/flexion strength.[17, 20] Maximal grip strength force in kilograms was measured two times using a handheld dynamometer on both hands (Jamar, Lafayette Instruments, Lafayette, IN). Maximal isokinetic leg extension/flexion torque was measured on both limbs with two trials of 5

repetitions at 60 and 180 degrees per second on an isokinetic dynamometer (Biodex Inc, Shirley, NY).

- Additional inflammatory markers were measured in exploratory analyses. These included novel (sCD163, sIL2R-α, sTNF-αR1) and traditional inflammatory markers (C-reactive protein). sCD163 is a biomarker of monocyte activation that is related to CVD risk and mortality in elders.[21] sIL2R -α is a marker of T cell activation that can result in accelerated loss of thymic function and adaptive immunity with chronic overexpression.[22] sTNF-αR1 has emerged as a superior surrogate marker of TNF-*alpha*, yielding data with considerably less analytical variability[23] — it is liberated from multiple cell types through the action of TNF-*alpha*, and therefore a biomarker of its activity.

Sample size

The sample size (N=300) was determined based on marginal comparisons (135 and 165/group) between each active intervention and placebo using a one-sided hypothesis tests at the 10% level. The goal was not to provide definitive evidence, but to rule out small effects that would have lower clinical value. For IL-6, there was 91% power to detect a difference if the difference (on the log scale) was at least 0.1625 (or a 15% difference). There was 66% power for a 10% difference and 99% power for a 20% difference. For 400 m walk speed, there was >99% power to detect >0.095 m/sec (a substantial meaningful change), and 86% power for a difference >0.038 m/sec (a small meaningful change) in walking speed. Lastly, there was 90% power to detect a difference of at least 0.99 SPPB units.

Treatment interventions and randomization

Interventions consisted of LO, ω-3, and their combination in a double-blind, placebo-controlled randomized trial. ω-3 fish oil and placebo (corn oil) were obtained from Epax, Aalesund, Norway and had identical shape, color, taste, and weight. Purity and composition of ω-3 was monitored with 13C NMR spectroscopy. LO and placebo were obtained from Almac Group, Souderton, PA. Three groups of potential participants were evaluated for the ENRGISE Pilot study. The first group consisted of potential participants who were not currently taking ω-3 fatty acids, fish oil (generic or specific, such as salmon, krill, or cod liver oil), flax, flaxseed oil or were not consuming > 2 servings of fatty fish per week (e.g. Salmon, Trout, Bluefish), but were taking either an ARB or ACE inhibitor. This group was randomized to placebo ω-3 or active ω-3. The second group consisted of potential participants who were not using an ACE inhibitor or an ARB but were taking ω-3. This group was randomized to placebo LO or to active LO. The third group consisted of people not using ω-3 or consuming other sources of polyunsaturated fatty acids as described above, an ARB, or an ACE inhibitor. This group was randomized to placebo LO/placebo ω-3, placebo LO/active ω-3, active LO/placebo ω-3, or active LO/active ω-3. Table 3 shows randomization strata and the number of participants in each group, according to baseline LO and ω-3 use. Randomization used a permuted block algorithm (with random block lengths) and was concealed via the secure web-based data management system.

Dose, titration plan and compliance

Participants randomized to the ω-3 arm began with 1.4 g/day (administered in 0.7 gram gel caps) until the 6-month follow-up visit. Each 0.7 grams of fish oil contained 400 mg eicosapentaenoic acid (EPA) and 200 mg docosahexaenoic acid (DHA). LO was obtained in 25 mg and 50 mg capsules. The LO and placebo were each encapsulated so that they were identical in shape, color, taste and weight. Participants randomized to LO began with 25 mg/day. If this dose was tolerated, based on the safety assessment that included reported symptoms and blood pressure at 1–2 weeks after the start of LO, then the dose of LO was increased to 50 mg/day. If there were no safety concerns, the dose of 50 mg/day continued until the 6-month visit. At the six month follow-up, the dose of ω-3 was increased to 2.8 g/day and LO was increased to 100 mg/day if the average of IL-6 measured at 3- and 6-month visits did not decrease by at least 40% vs. baseline (average of screening visits 1 and 2). The 40% threshold was selected based on findings of previous trials, which showed IL-6 reductions >50%.[24, 25] Compliance to the interventions was monitored with both pill counts at scheduled clinic visits and participant reports. For the latter, participants were asked how many times they were not able to take the study drug and asked to rate their ability to take it during in the past month (e.g. excellent, fair, poor).

Safety measures

Safety visits consisted of a follow-up visit after initiating or increasing study drug designed to specifically assess the safety of the medication increase. They were also scheduled to follow participants who required a dose reduction because of symptoms, abnormal findings or at the Medical Safety Officer's discretion. The safety visits were conducted within one to two weeks of a change in study medication and consisted of: 1. medical history follow-up, 2. medication inventory, 3. vitals check (blood pressure, pulse, weight & temperature), 4. safety labs relevant to the medication change, and 5. medication adherence. No drugs were dispensed at safety visits. Drug dispensing only occurred once laboratory results were obtained.

Measures to evaluate safety consisted of blood pressure, hemoglobin, serum glucose, renal function (eGFR), LDL cholesterol, and potassium at baseline and at 3, 6, and 9 months of follow-up. These measures were also collected at 1 to 2 weeks after randomization for participants randomized to LO/placebo. If serum potassium rose above 5.0mEq/L or eGFR dropped by more than 20% from baseline, LO treatment was discontinued. If blood pressure dropped to <90/50 mmHg (hypotension), or if participants experienced new symptoms of hypotension such as dizziness or pre-syncope, losartan dose was reduced or discontinued. For ω-3 fish oil, if there was a new onset of atrial fibrillation, or if hemoglobin decreased by >20%, or fasting glucose or LDL cholesterol significantly increased from a previous visit, ω-3 fish oil was discontinued. Laboratory tests and/or blood pressure were repeated whenever a dosage adjustment was made in response to an abnormal laboratory test result and/or blood pressure value. Blood testing was also repeated at the investigator or medical safety officer's discretion.

DISCUSSION

The ENRGISE Pilot Study was designed to test ability of anti-inflammatory interventions to improve or preserve walking ability in older adults with mobility impairments. The investigators selected interventions that are widely available, safe, tolerable, acceptable, and affordable for vulnerable older persons. Additionally, to maximize the anti-inflammatory effect and potential benefit on mobility, the investigators tested both individual and combination interventions.

Choice of potential candidate drugs

Potential candidate interventions were assessed using the following criteria: (1) excellent safety records, (2) the ability to reduce elevated IL-6 levels, (3) demonstrated benefits in improving physical performance, (4) considered innovative for affecting mobility outcomes, (5) tested in similar trials acting with different but complementary biological mechanisms, and (6) broadly available at low cost. A number of candidate interventions were excluded from consideration based on the *a priori* criteria listed in Table 4. Under the first criterion, anti-TNF-α agents (etanercept, infliximab, adalimumab),[26] anti-IL-6 agents (siltuximab),[27] anti-IL1β (canakinumab – no long-term safety data available),[28] and thiazolidinediones (rosiglitazone, pioglitazone)[29] were excluded based on risk of infections, liver toxicity, fluid overload, CVD, fractures, or possible cancer. Chloroquine and statins were excluded due to concerns of myotoxicity and lack of effect on walking speed for statins (criteria 1 and 5).[30] Corticosteroids, aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), and cyclooxygenase-2 (Cox-2) inhibitors were excluded for risk of bleeding,[31] gastrointestinal toxicity, and CV events for NSAIDs and COX-2 inhibitors.[32] Colchicine was excluded for risk of myotoxicity[33] and neuropathy (criteria 1 and 5).[34] Low-dose methotrexate is potentially safe and effective in lowering IL-6, but was not acceptable as it bears the stigma of being a “dangerous” anticancer drug. Criterion 2 excluded promising interventions, such as metformin, ghrelin, lactoferrin, oxytocin, salsalate, creatine, curcuma, probiotics, and resveratrol because of lack of clinical trial evidence for reducing IL-6. Metformin, while potentially promising, showed in the Diabetes Prevention Project a small reduction in CRP (-12%),[35] in part related to its modest weight-reducing effect. However other trials have shown no effect on IL-6[36] or CRP.[37] Despite the numerous potential anti-inflammatory interventions in the literature, most were deemed to have limited public health impact for prevention due to safety and high cost.

Only two potential interventions met the *a priori* inclusion criteria. First, ACE-Is and ARBs have shown excellent safety in large hypertension and heart failure trials in older persons. Among ACE-Is, perindopril[38] and enalapril[39] have demonstrated reductions in IL-6. Perindopril was shown to prevent physical function and walking speed declines in older persons[40] and to reduce CRP[41]. Regarding ARBs, most (except azilsartan) are known to reduce elevated IL-6 and were prioritized over ACE-I’s because they exhibit greater tolerability.[42] A second intervention meeting *a priori* inclusion criteria was ω-3 fish oil [43] and lipoic acid[44], which both show reduced IL-6 and CRP[43] in RCTs. In older women, ω-3 improved walking speed,[45] and muscle strength when supplemented with exercise.[46] LO[47] and ω-3,[48] also have supplementary effects on vasculature, coagulation, metabolism, and

skeletal muscle, all of which may benefit mobility (Figure). The combination of these interventions may provide added support regarding the relevance of their common anti-inflammatory effect on benefits of walking speed and ultimately mobility. The final decision was to prioritize LO and ω-3 (and their combination) due to established reductions of IL-6 by >40% in RCTs, excellent safety records, high tolerability, long history of trials, a shared complementary biological mechanism and their low cost (i.e. LO is 1/50 the cost of other ARBs).[24, 25]

Challenges and limitations

Pilot studies, by nature of their name, have inherent challenges and limitations. First, while we provide ample justification for choosing IL-6 as a marker of chronic low-grade inflammation, this marker may not provide the most robust association with the physical function outcomes. To evaluate this possibility, ENRGISE investigators assessed other markers of inflammation to compare sensitivity to change and association with the physical function outcomes of interest. The major challenges of the ENRGISE Pilot Study were identifying participants who met the IL-6 criteria while also having mobility impairments and yet were medically safe enough to participate. The joint prevalence of these criteria was largely unknown. Additionally, the MMD outcome required a large sample size to identify group differences.[49] If the MMD outcome proved to be too infrequent for a larger study, the proportion of participants who changed their walking speed by a small, yet clinically meaningful amount, was an alternative primary outcome for the main trial. Another important limitation of the design was that lowering of inflammation and improved or preserved walking speed may not be directly linked to each other. The interventions tested have a myriad of biological effects that could positively affect mobility (e.g. improved blood flow with ARBs, brain function, lipid profiles etc…) and these would be challenging to mechanistically separate from their effects on inflammation in the current design.

Conclusion

The ENRGISE Pilot study design and concept had several innovative approaches. First, it targeted chronic low-grade inflammation to achieve reduced inflammation that was hypothesized to result in improved mobility. The study enrolled an older population at high risk of mobility disability, who are often excluded from large RCTs. The intervention repurposed widely available inexpensive interventions (LO and ω-3) and tested them alone and in combination to maximize their effects on inflammation and mobility. The data collected in the ENRGISE Pilot study provides a large foundation of knowledge from which to plan for a full-scale trial.

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Elements of Financial/ Personal Conflicts	Manini		Anton		Beavers		Cauley		Espeland		Fielding		Kritchevsky		Leeuwenburgh		Lewis		Liu		McDermott	
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
Employment or Affiliation		X		X		X		X		X		X		X		X		X		X		X
Grants/Funds	X		X		X		X		X		X		X		X		X		X		X	
Honoraria		X		X		X		X		X		X		X		X		X		X		X

Elements of Financial/ Personal Conflicts	Manini		Anton		Beavers		Cauley		Espeland		Fielding		Kritchevsky		Leeuwenburgh		Lewis		Liu		McDermott	
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
Speaker Forum		X		X		X		X		X		X		X		X		X		X		X
Consultant		X		X		X		X		X		X		X		X		X		X		X
Stocks		X		X		X		X		X		X		X		X		X		X		X
Royalties		X		X		X		X		X		X		X		X		X		X		X
Expert Testimony		X		X		X		X		X		X		X		X		X		X		X
Board Member		X		X		X		X		X		X		X		X		X		X		X
Patents		X		X		X		X		X		X		X		X		X		X		X
Personal Relationship		X		X		X		X		X		X		X		X		X		X		X

Elements of Financial/ Personal Conflicts	Miller		Tracy		Walston		Radziszewsk		Lu		Stowe		Wu		Newman		Ambrosius		Pahor	
	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y	N
Employment or Affiliation		X		X		X		X		X		X		X		X		X		X
Grants/Funds	X		X		X		X		X		X		X		X		X		X	
Honoraria		X		X		X		X		X		X		X		X		X		X
Speaker Forum		X		X		X		X		X		X		X		X		X		X
Consultant		X		X		X		X		X		X		X		X		X		X
Stocks		X		X		X		X		X		X		X		X		X		X
Royalties		X		X		X		X		X		X		X		X		X		X
Expert Testimony		X		X		X		X		X		X		X		X		X		X
Board Member		X		X		X		X		X				X		X		X		X
Patents		X		X		X		X		X		X		X		X		X		X
Personal Relationship		X		X		X		X		X		X		X		X		X		X

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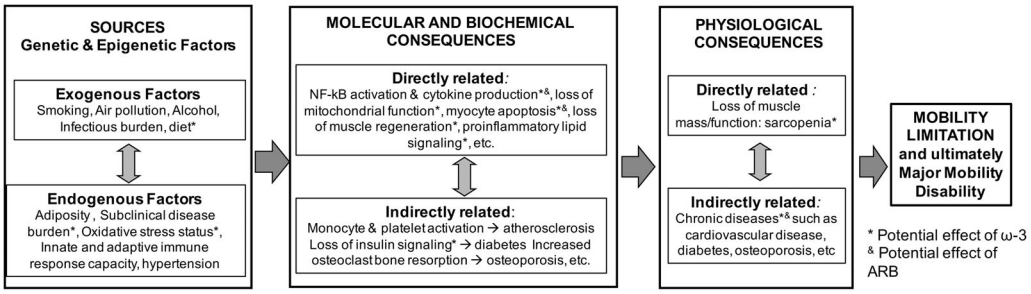

Figure.

Illustration of selected mechanisms potentially leading to progressive mobility disability that may be amenable to intervention using anti-inflammatory inventions being proposed by the ENRGISE Pilot study. The three main sources of inflammation in elders (genetic and epi genetic factors, exogenous factors and endogenous factors) combine to cause molecular and biochemical changes with important consequences, which in turn lead to physiological consequences and ultimately to mobility limitation and mortality.

Table 1

Inclusion and exclusion criteria for the ENRGISE Pilot Study

Inclusion Criteria

- Men and women age >70 years
- Self-reported difficulty walking ¼ of a mile or climbing a flight of stairs
- Walking speed <1 m/sec and >0.44 m/sec on the 4 m walk at usual pace. A walking speed of <0.44 m/sec would not be compatible with completing the 400 m walk in 15 min. (In the pilot phase we explore the feasibility of recruiting at least 50% of participants who have a baseline walking speed of <0.80 m/sec and >0.44 m/sec)
- Able to complete the 400 m walk test within 15 minutes without sitting or the help of another person and without a walker, a cane is allowed
- Blood level IL-6 >2.5 pg/ml and <30 pg/ml.
- Willingness to be randomized to the intervention groups

Exclusion criteria

- Failure or inability to provide informed consent
- Lives in a nursing home; persons living in assisted or independent housing are not excluded
- Self-reported inability to walk one block
- Significant cognitive impairment, defined as a known diagnosis of dementia, or a Mini-Mental State Exam (MMSE) score <24
- Unable to communicate because of severe hearing loss or speech disorder
- Neurological conditions that are causing impaired muscle function or mobility (may include stroke with residual paresis paralysis, neuropathy, Parkinson disease, or multiple sclerosis)
- Severe rheumatologic or orthopedic diseases, e.g., awaiting joint replacement, known active inflammatory or autoimmune disease (e.g. rheumatoid arthritis, lupus, Crohn's disease, HIV)
- Terminal illness with life expectancy less than 12 months
- Severe pulmonary disease, requiring either steroid pills or injections
- Other significant co-morbid disease that in the opinion of the field center PI would impair ability to participate in the trial, e.g. renal failure on hemodialysis, severe psychiatric disorder (e.g. bipolar, schizophrenia), excessive alcohol use (>14 drinks per week); drug addiction; treatment for cancer (radiation or chemotherapy) within the past 1 year; or other conditions
- Lives outside of the study site or is planning to move out of the area in next 1 year or leave the area for >3 months during the next year
- Exclusion criteria that apply only to those who receive losartan:
  - Intolerance or allergy to ARBs
  - Known bilateral renal artery stenosis or liver cirrhosis
  - Hypotension SBP<110 or DBP<60 mmHg
  - Serum potassium ≥5.4 mEq/L
  - Use of lithium salts
  - eGFR < 15
  - Congestive heart failure with ejection fraction < 40%
- Exclusion criteria that apply only to those who receive ω-3:
  - Intolerance or allergy to ω-3 or fish/shellfish
  - Omega-3 polyunsaturated fatty acids, fish oil (generic or specific, such as salmon, krill, or cod liver oil), flax or flaxseed oil
  - Fatty fish intake >2 servings per week on average (Salmon, Trout, Bluefish, Mackerel, Halibut, Herring, and Tuna)
  - History of paroxysmal or persistent atrial fibrillation
- To maintain blinding, those who are not eligible to receive any active treatment (ω-3 or losartan) are excluded

Temporary exclusion criteria

- Myocardial infarction, CABG, or valve replacement within past 6 months
- Pulmonary embolism or deep venous thrombosis within past 6 months
- Uncontrolled diabetes with recent weight loss, diabetic coma, or frequent insulin reactions
- Stroke, hip fracture, hip or knee replacement, or spinal surgery within past 4 months
- Physical therapy for gait, balance, or other lower extremity training within the past 2 months
- Severe hypertension, e.g., SBP > 200, or DBP > 110 mmHg
- Serum level of 25-OH Vitamin D <20 ng/ml (dropped as a criteria during the recruitment period)
- Hemoglobin <10 g/dL
- Participation in another intervention trial within 3 months; participation in an observational study may be permitted
- Current smoking within 6 months,
- Acute infection (urinary, respiratory, other) or hospitalization within 1 month
- Exclusion criteria that apply only to those who receive losartan:
  - Use of ACEI, ARB within 2 months
  - Use of aliskiren within 2 months in patients with type 2 diabetes or renal impairment with eGFR<60 [50]
  - Use of potassium sparing diuretics, other medications with potassium sparing properties (such as but not limited to spironolactone or eplerenone), potassium supplements, and salt substitutes containing potassium within 1 week
  - Transaminases >twice upper limit of normal to exclude participants with impaired liver function
- Exclusion criteria that apply only to those who receive ω-3:
  - Use of ω-3 polyunsaturated fatty acids, fish oil (generic or specific, such as salmon, krill, or cod liver oil), flax or flaxseed oil within 2 months
- To maintain blinding, those who are not eligible to receive any active treatment (ω-3 or losartan) are excluded

Table 2

Schedule of ENRGISE assessments and follow-up procedures

Visit type	Phone scr.	Scr. visit 1	Scr. visit 2	Baseline. visit	Safety*	3-Mo visit	Safety *	6-Mo visit	Safety*	9-Mo visit	Safety*	12-Mo visit	Safety*	Extra visit**
Basic eligibility screening	x													
Short consent and 4 m walk test		x												
IL-6		x	x			x&		x&		x&		x&		
Medical history			x											
MMSE			x											
Safety blood tests			x	x	x	x	x*	x	x*	x	x*		x*	x
Additional biomarkers				x		x		x				x		
Vital signs			x	x	x	x	x	x	x	x	x	x	x	x
Anthropometric measures				x		x		x		x		x		
Main Informed consent				x										
Physical performance measures^				x		x		x		x		x		
Medical history & adverse events				x	x	x	x	x	x	x	x	x	x	x
Questionnaires				x								x		
Dispense study drugs				x		x		x		x				
Assess compliance						x		x		x		x		
Proxy interview (if needed)						x		x		x		x		

*BP, and serum potassium and eGFR were measured about 1 week after the LO dose adjustment (potassium and eGFR were not measured if LO dose is reduced only for BP values).

**Extra visits if there were safety concerns.

&The IL-6 measure is excluded or postponed if there has been an acute illness within one month prior to the visit

^Physical performance measures include: 400 m walk test, short physical performance battery, maximal grip and leg extension/flexion strength

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Table 3

ENRGISE randomization strata according to medication use at baseline.

Strata according to medication use at baseline	ENRGISE Pilot N (%) Proposed	Randomization weights			
		Placebo LO Placebo ω-3	LO Placebo ω-3	Placebo LO ω-3	LO ω–3
1 No ω-3 (ACEI/ARB ok)	75 (25%)	0.4*	0	0.6*	0
2 No ACEI/ARB (ω-3 ok)	75 (25%)	0.4**	0.6**	0	0
3 No ACEI/ARB, No ω-3	150 (50%)	0.2	0.2	0.2	0.4
Overall	300 (100%)	0.3 (n=90)	0.25 (75)	0.25 (75)	0.2 (60)

* Did not receive placebo LO

** Did not receive placebo ω-3 as the use of the corresponding drugs is permitted at baseline

Table 4

Summary of selection criteria and candidate interventions

	Criteria					
Interventions	**1. Safe, tolerable, acceptable**	**2. IL-6 reduction**	**3. Physical performance**	**4. Innovation**	**5. Mechanism**	**6. Practical, affordable**
ACEIs, ARBs	+	+	+	+	+	+
ω-3	+	+	+	+	+	+
Anti-TNF-α, -IL6,-IL1; methotrexate thiazolidinediones	−	+	?	+	+	?
Statins, chloroquine, colchicine	−	+	− ?	+	−	+
Corticosteroids, aspirin, NSAIDs, cox-2 inhibitors	−	+	?	+	+	+
Metformin, fosinopril, ghrelin, lactoferrin, oxytocin, salsalate, curcuma, creatine, probiotics, resveratrol	+	− ?	− ?	+	+ ?	+

+ positive evidence, − negative evidence, ? evidence lacking