The ENRGISE Pilot Study Protocol

1. Project Title:

The ENRGISE Pilot Study

2. Investigators

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3. Abstract

Growing evidence shows that low-grade chronic inflammation, characterized by elevations in plasma C-reactive protein, tumor necrosis factor alpha, and particularly Interleukin-6 (IL-6), is an independent risk factor of disability, impaired mobility, and lower walking speed. Low-grade chronic inflammation is a modifiable risk factor. However, it is unknown whether interventions that reduce the levels of inflammatory markers *per se* improve mobility, or avert decline in mobility in older persons.

To address this gap in evidence we conduct the randomized clinical trial ENRGISE (ENabling Reduction of low-Grade Inflammation in SEniors) Pilot Study to test the ability of anti-inflammatory interventions for preventing major mobility disability by improving or preserving walking ability. We have maximized the public health impact by selecting interventions that are safe, tolerable, acceptable, and affordable for vulnerable older persons. Specifically, in this trial we test the efficacy vs. placebo of the angiotensin receptor blocker losartan and omega-3 polyunsaturated fatty acids in the form of fish oil, alone and in combination. Both angiotensin receptor blockers and omega-3 polyunsaturated fatty acids have shown to reduce IL-6 in clinical trials and preliminary data suggest that they may improve physical function.

We recruit older persons who are at risk for, or with, mobility impairment, as measured by slow gait speed and self-reported mobility difficulty, and who have elevated levels of IL-6, the marker most consistently associated with mobility limitations. Preliminary data regarding feasibility need to be gathered before such a trial can be effectively designed and implemented. We conduct The ENRGISE Pilot Study to assess the effects of the interventions on several inflammatory markers, walking speed, physical function and strength. This allows us to refine the design, recruitment yields, target population, adherence, retention, tolerability, sample-size, and cost for the main ENRGISE trial.

4. Specific aims

Growing evidence from our group and others shows 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),[1-7] is an independent risk factor for disability, impaired mobility, and slow walking speed.[1, 6-11] Low-grade chronic inflammation is a modifiable risk factor. However, it is unknown whether interventions that reduce the levels of inflammatory markers *per se* improve mobility, or avert decline in mobility in older persons.

To address this gap in evidence, we will conduct the main randomized clinical trial (RCT) **ENRGISE (ENabling Reduction of low-Grade Inflammation in SEniors)** to test the ability of anti-inflammatory interventions for improving or preserving walking ability. We have maximized the public health impact of the interventions by selecting interventions that are safe, tolerable, acceptable, and affordable for vulnerable older persons. Based on an extensive literature review, we test the efficacy of the angiotensin receptor blocker (ARB) losartan (LO) and omega-3 polyunsaturated fatty acids (ω-3) as fish oil. To maximize the anti-inflammatory effect and potential benefit on mobility, we explore both individual and combination interventions that target different, but complementary and potentially synergistic anti-inflammatory mechanisms.[12-15] We plan to recruit older persons at risk for, or with, mobility impairment, as measured by slow gait speed and self-reported mobility difficulty, and who have elevated blood levels of IL-6 (>2.5 pg/ml), the marker most consistently associated with mobility limitations.[1-7] Preliminary data on feasibility and information required for obtaining sample size estimates are needed to effectively design and implement the main trial. Thus, we implement the following **specific aims** for the ENRGISE Pilot Study:

a. Conduct a pilot RCT in 300 older persons at risk of mobility decline to assess: (i) compared with placebo, the effects of LO, ω-3, and LO+ω-3 on IL-6 and walking speed; (ii) the recruitment yields, the target population, adherence, retention, tolerability, sample-size, design, and cost for the main RCT; and (iii) the intra-subject variability of IL-6, and the dosage and safety of the interventions.
b. Measure novel and established inflammatory markers to:
    i. Characterize the effect of LO and ω-3 on these biomarkers and determine: (a) Are these cytokines/pathways impacted by the interventions? (b) Are the changes in these markers pre- vs. post-intervention less than, equivalent to, or greater than that of IL-6? and
    ii. Assess if these biomarkers yield independent information from IL-6 with respect to change in walking speed and/or provide benefit for screening participants for the main trial.

ENRGISE addresses critical public health issues regarding mobility disability prevention. We test the anti-inflammatory effects of widely available inexpensive interventions and their impact on mobility in a highly vulnerable population of older adults at risk of mobility disability.

5. Background

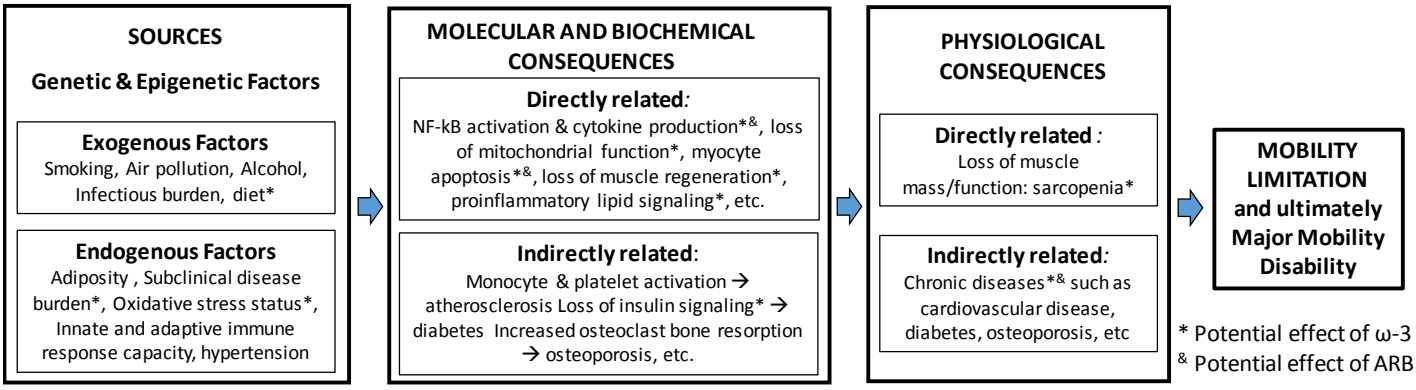
Mobility outcomes. Preserving mobility is central to maintaining a high quality of life and participation in activities to be fully independent in the community.[16, 17] Mobility limitation is associated with subsequent hospitalization, nursing home placement, increased healthcare costs, and death.[18-22]

Low-grade chronic inflammation and mobility outcomes. Acute inflammation is tightly regulated to promote healing and response to infection. In young, healthy persons, levels of inflammatory markers are low, while many older adults, even if apparently disease-free, have persistent, chronic, mild elevations of CRP, IL-6, TNF-α, and other markers. The degree of these elevations predicts disability, mobility limitations[1, 3] and mortality[23-25] independently of other risk factors. The basis for these elevations in older adults is not well understood. Smoking history and obesity,[26] especially visceral adiposity,[26] play a role, yet age itself remains the strongest predictor of these elevations in inflammatory markers.[1] Studies of the aging process suggest that senescent cells secrete IL-6, initiated by IL-1α and regulated by TGFβ.[27] Due to cell cycle arrest, senescent cells are less prone to malignant transformation, but may disrupt normal tissue function, promoting central adiposity, atherosclerotic plaque, and osteoporosis.[27, 28] Reduced renal function[29] and atherosclerosis[30, 31] may contribute

to the elevation of inflammatory markers, through decreased excretion and increased production of these markers. Thus, the "chronic inflammation" of aging may indicate the ongoing process of age-related damage and repair, and thus, contribute to the cumulative burden of disease-related damage, and cellular senescence.

The age-related dysregulation of immune function may have direct adverse consequences on physical function and promote disability by causing fatigue and loss of muscle strength.[7, 32, 33] CRP has been identified as an independent risk factor for cardiovascular disease (CVD) outcomes,[34-38] and clinical trials targeting the up-regulation of inflammation to reduce CVD events are currently underway.[39-41] In aging, IL-6 is thought to have a direct impact on fatigue and loss of muscle function. Injection of IL-6 produces acute and chronic atrophy of skeletal muscle in rats.[42] IL-6 levels tracked best with functional decline,[1, 4] though many inflammatory markers are involved and related to frailty.[43, 44] While conditions, such as myocardial infarction, stroke and arthritis, may contribute to disability and inflammation, the inflammatory state appears to have a direct downstream causal effect on disability. It follows that treatments that moderate the inflammatory pathway and reduce IL-6 may ameliorate mobility. Inflammatory pathways are tightly regulated so that manipulation could have adverse effects such as infection or delay of healing. Though exercise and weight loss both reduce IL-6 levels and improve mobility function providing circumstantial support,[45, 46] to our knowledge, the hypothesis that IL-6 reduction improves mobility function has not been directly tested.

Figure 5.1. The relation of inflammation to loss of physical function. The three main sources of inflammation in the elderly (genetic and epigenetic 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. These changes are complex with thousands of genes and other macromolecules involved; some examples of important pathways are listed. The potential impact of the chosen interventions are shown.



Detrimental mechanisms of action of inflammation in older persons. The sources and consequences of chronic inflammation are important for identifying promising interventions (Figure 5.1.). Inflammation in older persons results from: genes and their modifiers, the environment, and the *in situ* biochemical environment. Important exogenous environments include smoking, moderate (beneficial) and excessive alcohol consumption, and an anti-oxidative diet (beneficial). Pro-inflammatory endogenous factors include adiposity (especially visceral adiposity), subclinical disease burden, and oxidative stress, among others.

Regarding direct consequences, activation of NF-κB signaling increases muscle protein degradation and inhibits myogenesis,[47, 48] except in very late differentiation where it promotes homeostasis.[47] NLRP3-mediated IL-1β production plays a major role in mediating age-associated inflammation; ablation of this complex improves inflammation-associated detrimental changes with aging.[49] This inflammosome is activated by several products of damage including certain free fatty acids, free cholesterol, and reactive oxygen species.[49]

The cytokines TNF-α and IL-1β inhibit hormones critical for muscle homeostasis (e.g., growth hormone and IGF-1).[47] Circulating IL-6 is negatively associated with myosin heavy chain synthesis in patients with heart failure.[50] TNF-α activates muscle matrix metalloproteinase-9 (MMP-9), which degrades the extracellular matrix,[51] and can cause muscle cell apoptosis, which is attenuated by caloric restriction.[52] Cytokines also augment muscle infiltration by proinflammatory macrophage and lymphocytes,[47] including proinflammatory senescence-associated secretory phenotype (SASP) cells.[53] Aging muscle has accelerated mitochondria-mediated apoptosis and mitophagy linked to increased oxidative stress.[54]

ω-3 fatty acids are indicated to reduce triglyceride levels in adults with severe hypertriglyceridemia. ω-3 fatty acids are also anti-inflammatory [55] via a specific ω-3 fatty acid receptor, GP120 [56] which blocks NF-κB and JNK signaling, counter to saturated fatty acid-mediated proinflammatory TLR2 signaling.[57] Saturated fats

are generally pro-inflammatory,[58] and can cause a decline in muscle protein synthesis via ER stress.[59] ω-3 supplementation down-regulates inflammation[60] in part through decreased TNF-α expression and ω-3 fatty acids are likely to improve mTOR signaling, thereby stimulating muscle protein synthesis in humans.[61] A meta-analysis of several RCTs has shown that ω-3 reduce both IL-6 and CRP in chronic non-autoimmune disease.[62]

ARBs are primarily indicated for the treatment of hypertension. The ARB LO is also a partial PPAR-γ agonist,[63] yielding potent anti-inflammatory effects.[64] LO reduces IL-6 in human[65] and animal studies,[66] possibly through PPAR-γ/AMPK agonism, blockage of proinflammatory angiotensin II AT1 signaling, or both.[64, 67] ARBs mitigate lipopolysaccharide -mediated inflammation, and inhibit TNF-α-mediated endothelial Receptor for Advanced Glycosylation Endproduct (RAGE) expression.[68] LO has been shown to improve skeletal muscle related activity measures in older mice.[66, 69] In summary, inflammation plays a major role in the loss of physical function through a wide variety of mechanisms, many of which offer interventional targets.

All drugs have multiple effects. LO[63, 67, 70] and ω-3,[71, 72] in addition to averting inflammation, also affect vasculature, coagulation, metabolism, and skeletal muscle, all of which may benefit mobility (Figure 5.1.). Testing these two different interventions that avert inflammation may provide added support regarding the relevance of their common anti-inflammatory effect in possibly benefiting walking speed and ultimately mobility.

Selection of anti-inflammatory interventions. A broad range of pharmacological, nutritional, and behavioral interventions have potential efficacy in reducing low grade chronic inflammation.[39, 74] To maximize the public health impact of ENRGISE, we set the criteria outlined in Table 5.1. and 5.2. for prioritizing interventions. Based on the selection criteria and the evidence reviewed below, in the pilot phase we test **LO and ω-3**. Both have excellent safety records, are tolerable and acceptable, reduce elevated IL-6, have shown benefits in improving physical performance, have not been tested in similar trials, act with different but complementary biological mechanisms,[12-15] do not negatively interfere with neuromuscular metabolism, and are broadly available at low cost. **ω-3 reduced IL-6 by >50%** in RCTs[75, 76] and **ARBs reduced IL-6 by >40%[77, 78] and by 58%[79]** in RCTs. In Health ABC[1] and EPESE,[2] we have found that **a 50% lower IL-6 plasma level was associated with approximately 50% lower risk of mobility disability** over 2.5 and 4 years of follow-up, respectively. Change in IL-6 induced by LO and ω-3 may depend on the target population, baseline IL-6 level, dosage, compliance, and duration of treatment. Thus, the pilot study is of key importance to probe their anti-inflammatory effects in the ENRGISE population.

Table 5.1. Criteria for prioritizing the selection of the ENRGISE interventions

1.	**Safety, tolerability and acceptability** are key criteria. Vulnerable older persons use multiple drugs and have multiple comorbidities, and thus, are at high risk of adverse drug reactions. Newer drugs are often tested in younger or middle age adults for the treatment of a single condition and therefore, their safety and tolerability in older persons is not fully known.[73] Furthermore, for the prevention of mobility limitations, vulnerable or frail older persons may not be willing to take, and their providers may not be willing to prescribe drugs that bear a risk of severe adverse events. Finally, we exclude interventions that may negatively affect skeletal muscle or neuromuscular metabolism.
2.	**Reduction of IL-6** and possibly CRP in clinical trials is our primary criterion.
3.	**Benefit on physical performance and/or skeletal muscle** is a relevant, but not necessary criterion.
4.	**Innovation**. We have prioritized interventions that have not been, or are not being tested in RCTs for preventing mobility limitations.
5.	**Biological mechanisms** are considered to prioritize interventions that target different inflammatory mechanisms or may have synergistic effects. We exclude interventions that may negatively affect skeletal muscle metabolism.
6.	**Practical and affordable** for implementation in the US health care system. Cost is a major factor for this criterion to maximize the public health impact of the trial

Inclusion of potential candidate interventions (see Table 5.2.)

Angiotensin Converting Enzyme Inhibitors (ACEIs) and ARBs have shown excellent safety in large hypertension and heart failure trials in older persons,[80-84] Among ACEIs, perindopril[85, 86] and enalapril[65] have demonstrated reductions in IL-6. Perindopril has shown to prevent physical function and walking speed decline in older persons,[87] to reduce CRP,[88] and to have a favorable safety profile.[89] Dual angiotensin blockade with ACEIs and ARB results in higher risk of adverse events,[90] thus, we do not recommend to combine ACEIs and ARBs. Virtually all ARBs, except azilsartan, have been shown to reduce elevated IL-6.[65, 77, 91-102] Telmisartan improved walking distance in patients with peripheral artery disease[103] and running endurance in mice.[15] In older mice LO reduced IL-6 and improved physical performance.[66] We prioritize ARBs because of greater tolerability than ACEIs,[83, 84, 104] and among ARBs, we prioritize LO due to its low cost (1/50 that of other ARBs). Among nutritionals, ω-3[62] and lipoic acid[92, 105] reduce IL-6[62, 92, 105] and CRP[62, 106] in RCTs. The evidence of the

anti-inflammatory effect is particularly strong for ω-3.[62] In older women, ω-3 improved walking speed,[107] and strength when supplemented to exercise.[108] ω-3 are widely available at low cost primarily in the form of fish oil. Among behavioral interventions, compared with Western diet, the Mediterranean diet (MED) reduces IL-6[109-113] and CRP.[109, 110, 112, 113] Olive oil contains oleocanthal acting as a natural anti-inflammatory compound that has a potency and profile similar to that of ibuprofen.[55, 114] MED is associated with better physical performance, higher walking speed and lower risk of frailty.[115-118] MED is promising, however, after consulting with diet experts, including Dr. Linda Van Horn at NWU, Dr. Mara Vitolins at WFSM, and Dr. Stephen Anton at UF, it was concluded that diet interventions are complex and too resource intensive to be considered in this context, primarily due to the need for dietary counseling. Additionally, the most successful MED trials were conducted in Spain and Italy,[110, 113, 119] where MED is part of the local traditions. It is doubtful whether MED could be successfully imported in the ENRGISE older frail US population within the available resources of this trial. Thus, after careful consideration, we did not prioritize diet interventions.

Exclusion of potential candidate interventions (see Table 5.2.)

Criterion #1 excludes anti-TNF-α agents (etanercept, infliximab, adalimumab),[120] anti-IL-6 agents (siltuximab),[121] anti-IL1β (canakinumab – no long-term safety data available),[122] and thiazolidinediones (rosiglitazone, pioglitazone)[123] for risk of infections, liver toxicity, fluid overload, CVD, fractures, or possible cancer. Chloroquine and statins are excluded due to concerns of myotoxicity[124-126] and lack of effect on walking speed for statins (Criteria #1, #5).[127] Corticosteroids, aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), and cyclooxygenase-2 (Cox-2) inhibitors are excluded for risk of bleeding,[128-132] gastrointestinal toxicity, and CV events for NSAIDs and COX-2 inhibitors.[133] Colchicine is excluded for risk of myotoxicity[134, 135] and neuropathy (Criteria #1, #5).[135] Low-dose methotrexate is potentially safe and effective in lowering IL-6. But, we found it is not acceptable by the older target population. A pilot study to assess effects on walking speed did not randomize a single participant, primarily because of refusal to take methotrexate expressed by participants or their health providers (section 3.d.3). Methotrexate bears the stigma of a "dangerous" anticancer drug in these older people. Many of the above excluded drugs would likely face similar non-acceptability problems; even if they were effective, they would have limited public health impact for prevention, due to high cost and risk of adverse effects.

Criterion #2 excludes promising interventions, such as metformin, ghrelin, lactoferrin, oxytocin, salsalate, creatine, curcuma, probiotics, and resveratrol because of lack of clinical trial evidence of reducing IL-6. Metformin, while potentially promising, has shown in the DPP a small reduction in CRP (-12%),[136] in part related to its modest weight-reducing effect, however other trials have shown no effect on IL-6[137] or CRP.[138-142]

Criterion #4. Physical activity and weight loss have shown in multiple RCTs both reduction of IL-6 and prevention of mobility limitations,[45, 46, 143-147] and thus, are not prioritized as innovative approaches.

Table 5.2. Summary of selection criteria and candidate interventions (see details in the text)

Criteria / Interventions	1. Safe, tolerable, acceptable	2. IL-6 reduction	3. Physical performance	4. Innovation	5. Mechanism	6. Practical, affordable
ACEIs, ARBs	+	+	+	+	+	+
ω-3	+	+	+	+	+	+
Mediterranean diet	+	+	+	+	+	-
Physical activity, weight loss	+	+	+	-	+	+?
Vitamin D	+	+	+	-	+	+
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

Innovation and strengths. The following features of ENRGISE represent innovative approaches:

- Targeting chronic low grade inflammation to achieve reduced inflammation and reduced mobility limitations
- Targeting an older population at high risk of mobility disability, which is often excluded from large RCTs
- Repurposing widely available inexpensive interventions (LO and ω-3)
- Testing LO and ω-3 in combination to maximize their effects on inflammation and mobility
- The use of several adaptive features in design and stratified screening for ENRGISE pilot study

- The assessment of novel inflammatory markers in ENRGISE pilot study

6. Research Plan

6.1. Design. We test LO, ω-3 and their combination in a double-blind, placebo-controlled RCT (The ENRGISE Pilot Study). Randomization is concealed via the secure web-based data management system. We use a permuted block algorithm (with random block lengths).

LO and ω-3 are considered safe in elders.[80-84, 148, 149] We conduct this pilot RCT to (a) decide whether we should proceed to the main ENRIGISE trial with 1 or 2 interventions, and (b) if two interventions are warranted whether we should proceed as a three-arm (without the combination group) or a 2x2 factorial study. The pilot is designed to achieve the following goals: (1) estimate recruitment yields including percent consenting; (2) confirm safety and tolerability; (3) assess the impact of interventions on IL-6 and walking speed as well as the secondary outcomes of Short Physical Performance Battery (SPPB), frailty, and muscle strength; (4) evaluate the strength of relationship between the change in IL-6 with the change in walking speed; (5) allow selection of the interventions that demonstrate high participant compliance and retention; (6) collect data on outcomes of major mobility disability and other mobility measures; (7) support the decision to proceed with a factorial or parallel design; (8) examine intra-subject variability of IL-6; (9) explore the impact of the interventions on additional biomarkers, their relationship with walking speed and their impact on recruitment and effect size estimates; and (10) estimate the cost of the main trial.

Table 6.1. Randomization strata according to medication use at baseline – Total N=300

Strata according to medication use at baseline	LIFE N (%) Observed	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)	637 (44.1)	75 (25%)	0.4*	0	0.6*	0
2 No ACEI/ARB (ω-3 ok)	154 (10.7)	75 (25%)	0.4**	0.6**	0	0
3 No ACEI/ARB, No ω-3	655 (45.3)	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)

* Does not receive placebo LO, and ** does no not receive placebo ω-3 as the use of the corresponding drugs is permitted at baseline

Recruitment is planned to last 1 year and each participant is being followed for 1 year. To assess recruitment yields according to different targets, we stratify on ACEI/ARB and baseline ω-3 use. The first stratum (people not using ω-3) is randomized to placebo or ω-3. The second stratum (people not using ACEI or ARB) is randomized to placebo or LO. The third stratum (people not using ACEI, ARB, or ω-3) is randomized to placebo, LO, ω-3, or the combination. The original ENRGISE targets, based on the estimated proportions in LIFE are presented in Table 6.1. (We note that during the ENRGISE DSMB's August 23, 2016 phone call, the DSMB approved the investigators' request to lift the upper limit on the number recruited into stratum 1. In the text which follows, the original sample sizes remain as we do not know how many we will recruit into each stratum. The randomization weights will remain unchanged.) We expect to have a slight imbalance in the overall numbers within each treatment group of 90, 75, 75, and 60. Oversampling of the control group allows us to better estimate parameters associated with IL-6, walking speed, and major mobility disability in that group; this is important for planning the sample-size of the main trial. Note that anybody eligible for stratum 3 is also eligible for strata 1 and 2. To achieve overall balance between the two single interventions, we focus attention on having equal numbers in strata 1 and 2 but do not worry if either is less than 25% of the total as randomizing additional people in strata 3 provides similar information. If 20% were in each of strata 1 and 2, the overall weights would be 0.28, 0.24, 0.24, and 0.24; if 15%, then 0.26, 0.23, 0.23, and 0.28. Both are close to Table 6.1. and do not markedly reduce power for any comparison.

6.2. Interventions and compliance. .

6.2.a. ω-3 fish oil and placebo (corn oil) are obtained by Epax in 0.7 g gel caps and they have identical shape, color, taste and weight. Each 0.7 g of fish oil ω-3 contains 400 mg eicosapentaenoic acid (EPA) and 200 mg docosahexaenoic acid (DHA). We start with 1.4 g/day of fish oil and continue until the 6 month visit. If the average of IL-6 measured at 3- and 6-month visits does not **decrease by ≥40%** vs. baseline (average of screening visits 1 and 2), we increase the dose to 2.8 g/day. To limit the effect of within subject variation, we use the average of 2 measures. If there is an acute illness within one month prior to the visit, the 3-month or the 6-month IL-6 measure is postponed or excluded from titration algorithms. In previous RCTs, ω-3 reduced IL-6 by >50% using 1 to 2 g/day of fish oil.[75, 76] In other trials in elders, fish oil was used in comparable doses

ranging from 0.6 to 2.3 g/day.[62]

6.2.a.1. Risks and risk protection and dose adjustments related to administration of ω-3

There are no contraindications to the use of ω-3 fish oil, except intolerance or allergy to fish oil. ω-3 are considered very safe and produce only mild adverse effects involving gastrointestinal upset and fishy aftertaste.[148, 149] FDA has affirming menhaden fish oil as **Generally Recognized As Safe (GRAS)** under 21 CFR 4 184.1472 provided that the daily intake of EPA and DHA combined do not exceed 3 g per person per day. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1472 and http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/SCOGS/default.htm accessed on 9/11/2014.

ω-3 may rarely cause clinical or subclinical bleeding, worsening of glycemic control in people with impaired glucose tolerance, rise in LDL cholesterol, and possibly hypotension when combined with antihypertensive treatment.

We start with a dose of 1.4 g/day of ω-3 fish oil. Adverse effects, such as gastrointestinal discomfort are monitored. Dose will be adjusted as below based on serum glucose, LDL cholesterol and hemoglobin measured at baseline and at month 3, 6, and 9. ω-3 fish oil dose will be decreased or discontinued if any of the following criteria are met at follow-up:

- New onset paroxysmal or persistent atrial fibrillation
- Hemoglobin decreases by ≥20% from baseline (dose will be discontinued),
- The participant is not diabetic and fasting glucose is > 126 AND has increased by >30% from baseline,
- The participant is diabetic and fasting glucose > 300,
- Baseline LDL > 130 AND increases by ≥ 20% from baseline,
- The participant is taking a blood pressure lowering agent OR is also participating in the Losartan arm of the study AND blood pressure is <90/50, or
- Participant does not tolerate the fish oil.

The principal investigator/study physician at each site has the discretion to opt out of ω-3 fish oil dose adjustments due to glucose and/or LDL levels.

We increase the dose of ω-3 fish oil to 2.8 g/day if the average of IL-6 measured at 3- and 6-month visits has not **decreased by at least 40%** vs. baseline (average of screening visits 1 and 2). The 40% threshold is selected based on previous trials with ω-3 fatty acids. ω-3 fatty acids have been shown to reduce IL-6 by >50% in RCTs[75, 76] and to be consistent with the threshold used below for losartan. To minimize the impact of outliers due to acute illness, the 3-month or the 6-month IL-6 measure are excluded or postponed if there is an acute illness within one month prior to the visit. In the rationale for dose titration we use the following assumptions:

1. IL-6 biovariation as a CV is ~30%. This is a reasonable estimate based on our own estimates (unpublished data) and those of others.[150, 151] This means that a SD of each person's day to day variability is about 30% of the mean value.
2. We have two measures at baseline that can be averaged to yield a reasonable estimate of each person's average value.
3. For dose adjustment we use IL-6 measured at 3 months and 6 months.
4. The half-life of IL-6 is extremely short (<30 minutes), so response to interventions is essentially coincident to the effect of the interventions.
5. We screen for outliers by excluding participants who experienced an acute illness within a month of the visit

6.2.b. LO and placebo (cellulose based) are obtained in 25 mg and 50 mg capsules. The shell capsules are cellulose based. Placebo and LO have identical shape, color, taste and weight. We start with 25 mg/day, if tolerated, we step up to 50 mg/day. If there are no safety concerns, we continue with 50 mg/day until the 6 month visit. If the average of IL-6 measured at 3- and 6-month visits does not **decrease by ≥40%** vs. baseline (average of screening visits 1 and 2), we increase the dose to 100 mg/day. Equivalent doses of ARBs have shown to reduce IL-6 by >40%[77, 78] and by 58%[79] in RCTs.

6.2.b.1. Risks, risk protection and dose adjustment related to administration of the ARB Losartan.
ARBs are reported to have a side effect profile indistinguishable from that of placebo but adverse reactions can occur rarely. Losartan has been safely used in large long-term clinical trials in older persons with congestive heart failure.[83, 84] ARBs are particularly well tolerated with very few side effects. Because ARBs do not influence kinin metabolism, dry cough is not seen and angioedema is very rare. Impairment in renal function may arise in patients with bilateral renal artery stenosis. Renal failure, reversible on discontinuation of ARB, may be precipitated.

- Hyperkalemia due to potassium retention mediated by reduction of aldosterone. Rare except in renal impairment.
- Impairment of renal function. Caution if bilateral renal artery stenosis suspected.
- Dizziness and syncope. Rare but may be precipitated by volume depletion.
- Angioedema. Very rare.
- Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Including Selective Cyclooxygenase-2 Inhibitors (COX-2 Inhibitors): In patients who are elderly, volume-depleted (including those on diuretic therapy), or with compromised renal function, co-administration of NSAIDs, including selective COX-2 inhibitors, with angiotensin II receptor antagonists (including losartan) may result in deterioration of renal function, including possible acute renal failure. These effects are usually reversible. We monitor renal function periodically in patients receiving losartan and NSAID therapy as outlined below.

A full list of potential side effects of losartan can be found in the current package insert.

6.3. Safety dose titration. ARBs produce blood pressure reductions similar to those seen with ACE inhibitors and other antihypertensive classes. These drugs vary in efficacy and duration of action but all are recommended for once daily dosing. Like ACE inhibitors, duration of antihypertensive effects is dose-dependent; therefore, smooth blood pressure control over 24 hours is most likely at the maximum recommended dose. To avoid precipitous initial fall in blood pressure or decline in renal function, we start therapy with the lower dose of losartan (25 mg/day). If there are no safety concerns, the dose is increased to 50 mg/day and safety is again checked in about 1 week. To identify abrupt reduction in renal function that warrants drug dose reduction and to avoid dangerous hyperkalemia, we measure eGFR, potassium, and blood pressure before and soon (approximately 1 week) after starting losartan and after any dose adjustment. However, dose adjustments downward that are not related to renal function or hyperkalemia will not require a short-term follow-up blood draw.
In general, losartan dose will be maintained if ALL of the following criteria are met:

- Blood pressure is ≥ 100/50 and < 110/50,
- Potassium < 5.5,
- eGFR has NOT declined by ≥ 20% from baseline, and
- No symptoms of lightheadedness or dizziness.

Losartan dose will be decreased if ALL of the following criteria are met:

- Blood pressure is ≥ 90/50 and < 100/50,
- Potassium < 5.5,
- eGFR has NOT declined by ≥ 20% from baseline, and
- No symptoms of lightheadedness or dizziness.

Losartan dose will be stopped if ANY of the following criteria are met:

- Blood pressure < 90/50,
- Potassium ≥ 5.5,
- eGFR decline ≥ 20% from baseline, or
- Symptoms of dizziness, lightheadedness, or syncope.

The principal investigator/study physician at each site has discretion to alter the losartan titration schedule on a case by case basis.

We increase the dose of losartan to 100 mg/day if the average of IL-6 measured at 3- and 6-month visits is not **decreased by at least 40%** vs. baseline (average of screening visits 1 and 2). The 40% threshold

was selected based on previous trials with ARBs. ARBs have been shown to reduce IL-6 by >40%[77, 78] and by 58%[79] in RCTs, and to be consistent with the threshold used above for ω-3 fish oil. To minimize the impact of outliers due to acute illness, the 3-month or the 6-month IL-6 measure are excluded or postponed if there is acute illness within one month prior to the visit.

As part of the safety monitoring of the ENRGISE interventions, we measure blood pressure, blood hemoglobin, serum glucose, eGFR, LDL cholesterol, and potassium at baseline and at 3, 6, and 9 months of follow-up. In addition for those in the losartan arm of the trial, blood pressure, serum potassium, and eGFR, are measured about 1 week after randomization and about 1 week after any losartan dosage adjustment. However, serum potassium and eGFR are not measured one week after a downward dose adjustment that was not based on potassium or eGFR values.

6.4. The Investigational New Drug (IND) Per requirement of the Institutional Review Board, #IND124103 was submitted and approved by the FDA with the mention "STUDY MAY PROCEED"

6.5. Compliance

To measure compliance with LO and ω-3, we use dual methods involving both pill count[152] and participant recall.[153] Pills are counted at each study visit.

6.6. Recruitment – inclusion/ exclusion criteria - (details in Table 6.2.)

We identify men and women aged ≥70 years who are **able to complete the 400 m walk** at baseline, and are at **high risk of mobility disability** assessed by

- Self-reported difficulty walking ¼ mile or climbing a flight of stairs,
- Usual walking speed <1 m/sec and >0.44 m/sec on the 4 m walk (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),[22, 154, 155] and
- Chronic low-grade inflammation measured by IL-6 >2.5 pg/ml, the threshold we have found for increased risk of mobility limitation,[1, 2] and <30 pg/ml to exclude high grade inflammation. To minimize within person variability,[150, 156] IL-6 is the average of 2 measures taken at screening visits, with the first measure being >2.3 and <30 pg/ml, and the average of the two measures >2.5 and <30 pg/ml.

We exclude subjects with acute, autoimmune and HIV illnesses and causes of low walking speed that may be related to neurological conditions, severe arthritis, or low vitamin D. Other exclusion criteria reflect conditions that may interfere with the conduct of the study, interventions or assessments. Those taking ACEI or ARB are excluded from the LO randomization and those eating>2 servings/week of fish in the past year or taking fish oil are excluded from the ω-3 randomization. We recruit from the community. We use recruitment strategies successfully applied in LIFE,[157] LIFE-Pilot,[158] and TTrial,[159] which include primarily mass mailing, and newspaper, radio and TV ads and explore alternative recruitment strategies, such as electronic medical records to identify patients not taking study drugs and assess whether that strategy is effective in lieu of or in addition to mass mailing.

Table 6.2. Inclusion and exclusion criteria

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 (<23 for racial/ethnic minorities or participants with less than 9 years of education)
- 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 ≥5.0 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
    - Fatty fish intake >2 servings per week on average
    - 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;
- 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 [161]
    - 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 within 2 months

To maintain blinding, those who are not eligible to receive any active treatment (ω-3 or losartan) are excluded

6.7. Outcomes

6.7.a. Primary Outcomes

IL-6 and walking speed during the 400 m walk test are the main outcomes of the pilot study as outlined below.

The 400 m walk test at usual pace is used.[143] **Major mobility disability (MMD),**[143, 162] defined as inability to walk ¼ mile or 400 m, is measured in the pilot study and is our preferred primary outcome for the main trial. MMD is of major public health significance. Ability to walk ¼ mile is measured in the US census[163] and in most epidemiologic surveys.[18] The MMD outcome based on the 400 m walk test is a feasible, objective, reliable,[164] well-validated and important clinical and public health outcome in older people,[22, 143, 162] which has been

successfully implemented in the LIFE-Pilot and LIFE.[165, 166] We have shown it to be a more efficient outcome for clinical trials than self-reported disability or the Short Physical Performance Battery (SPPB).[167] Public health agencies use ability to walk ¼ mile or 400 m to define need and policy impact of interventions.[18] Finally, people reporting the inability to walk 400 m incur higher health care costs of $4,000 per person per year, compared with those not reporting inability to walk 400 m.[18-22] MMD is operationalized as the inability to complete a 400 m walk test within 15 min without sitting or help of another person or walker.[162] Completing the walk in >15 min would be in an extremely slow pace (<0.45 m/sec), which is of little utility in daily life.[168] A higher cut point (30 or 60 min), makes the assessment impractical and does not add to the clinical significance of the outcome. The time to walk 400 meters and the ability to complete the test provide data to test effects of the interventions resulting from both attenuation of decline and increase in walking speed. We hypothesize that the interventions reduce the risk of reaching the MMD outcome.

Non-completion of this test can be addressed by adjudicating MMD based on objective inability to walk 4 m in≤10 sec, or self- or proxy-reported inability to walk across a room.

6.7.b. Secondary and exploratory outcomes and measures

SPPB. A low score on the SPPB based on 4 m walk, balance & chair stands tests is a risk factor for disability, institutionalization, morbidity and mortality in initially non-disabled older persons.[1-3] The summary score and components of the SPPB have good reliability (ICCs range from 0.88 to 0.92).[4,5]

Frailty is a state of increased vulnerability to endogenous and exogenous stressors.[169, 170] Frailty involves fatigue, weight loss, infections, balance, strength and gait impairment leading to an increased risk of falls, delirium, and disability. In addition frailty is associated with inflammation[43] and thus it may be averted by anti-inflammatory interventions. We characterize frailty with Fried criteria developed by Fried et al. that employ self-reported exhaustion, unintentional weight loss, low energy expenditure, slow gait speed, and weak grip strength.[171] Those with ≥3 of the 5 factors are judged to be frail, those with 1 or 2 factors as pre-frail, and those with no factors as non-frail.

Isometric hand grip strength is a commonly used measure of upper body skeletal muscle function and has been widely used as a general indicator of functional status.[172, 173] Higher IL-6 levels are associated with lower grip strength.[3, 7, 174] Thus, reducing IL-6 with LO and ω-3 may result is higher grip strength vs. placebo. Grip strength in both hands is measured as we have successfully applied in LIFE.[162]

Table 6.3. Schedule of screening, assessments and follow-up procedures in the pilot trial

Visit type	Phone scr.	Scr. visit 1	Scr. visit 2	Base. 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		x		
Medical history update & 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 are measured about 1 week after the LO dose adjustment (potassium and eGFR are not measured if LO dose is reduced only for BP values). ** Extra visits if there are safety concerns. & The IL-6 measure is excluded or postponed if there has been an acute illness within one month prior to the visit

Isokinetic dynamometry of the knee extensors and flexors. Participants complete this measure at baseline and 12 months. Observational studies have shown associations between knee extension isokinetic strength and physical functioning, disability and mortality.[176] The reliability of this measure is generally excellent.[177, 178] In addition, several randomized trials in older mobility limited populations have shown this measure to be responsive to therapeutic interventions.[179, 180]

The Short Form (SF-36) Health Survey [181-184] is administered to assess the general health status as

Participant Reported Outcomes (PRO).

6.8. Screening, assessments and follow-up (Table 6.3.)

Potential participants are screened by telephone interviews to assess the main inclusion/exclusion criteria. Those who qualify are invited for the first screening visit during which a brief informed consent is obtained and the 4 m walk at usual pace is administered. Blood level of IL-6 is tested in those with a walking speed <1 m/sec and >0.44 m/sec. Participants with qualifying IL-6 levels are invited for the second screening visit to review medical history and current medications, complete blood testing (vitamin D, CBC, chemical panel, and second IL-6 measure), vital signs (blood pressure, pulse and temperature), 400 m walk, and the Mini Mental State Examination (MMSE).[185] The study medical safety officer will review the participant's health history, medication use, vital signs, and laboratory test results, and clear for randomization according to the study inclusion and exclusion criteria. If there are no safety concerns, participants attend the baseline visit during which the following is completed: full informed consent, questions to assess frailty,[171] blood collection for labs, vital signs and anthropometric measurements, the SPPB, isokinetic dynamometry of the knee extensors and flexors, grip strength, and questionnaires. Validated Minnesota physical activity[186], depressive symptoms (CES-D)[175], patient reported outcomes (SF-36), and fatigability[198] questionnaires are completed at the baseline and 12 month visit. The physical activity results are used as potential modifiers in the data analyses. If there are no exclusion criteria identified, the participants are randomized, and the study drugs are dispensed. The baseline measures are repeated as outlined in Table 6.3. BP&P, blood tests and adverse events are measured about 1 week after randomization and after each visit when the LO dose is modified. Medication dose is adjusted as outlined in the above titration and safety algorithms.

6.9. Measure of additional biomarkers Inflammation, characterized by higher blood cytokine levels related to the activation of innate immunity and other systems (coagulation), is part of the aging process, plays a role in virtually all diseases, and contributes to mobility limitations.[1] While it is known that inflammation markers predict these outcomes and mortality, little is known about how anti-inflammatory interventions affect the plasma cytokine levels, or associated pathways. Thus, we measure novel and established markers of inflammation and aging at baseline, and follow-up, and relate these to both change in IL-6 and change in walking speed to:

(1) Characterize the effect of the interventions on these cytokines: (1.a.) Are these cytokines/pathways impacted by the interventions? (1.b) Are the changes in these markers pre- vs. post-intervention less than, equivalent to, or greater than that of IL-6?

(2) Assess if these biomarkers yield independent information from IL-6 with respect to the change in walking speed and/or provide benefit for screening participants in the main ENRGISE trial.

6.10 Data analyses and power for the ENRGISE Pilot Study and biomarkers assays

The data analyses and power section is presented in the order by which the aims are likely to be achieved rather than the order in which the aims are stated elsewhere in the protocol.

6.10.a. Feasibility and recruitment

Feasibility of recruitment and identifying the study population are contained in Aim a (ii). In the ENRGISE Pilot Study we will use one-sided tests/confidence intervals because we wish to rule out conditions that would make the main study infeasible. Having a higher than hypothesized safe tolerable rate, higher yield for recruitment, or stronger relationship between the changes in IL-6 and walking speed would simply make the follow-up study easier to implement. For efficacy, we use tests at the 10% significance level;[187, 188] this is consistent with the FDA's aims for phase 2 versus phase 3 studies.[189, 190] No formal adjustment for multiple comparisons is planned for the pilot study.

We assess the recruitment yields in strata 1-3 presented in Table 6.1 and the proportions of people who tolerate LO and ω-3. One-sided exact confidence intervals of these proportions are calculated. If we need to screen 300 people to recruit 75 into strata 1 (People not using ω-3) or 2 (People not using ACEI or ARB) (25% yield), the 95% one-sided CI would set the lower bound of our recruitment yield at 20.9% using a Clopper-Pearson interval[191] and 8.3% if we need to screen 750 (10% yield). For stratum 3 (People not using ACEI, ARB, or ω-3) (target 150), these lower limits would be 22.1% (25% yield) or 8.8% (10% yield). Note that those eligible for stratum 3 are also eligible for strata 1 and 2; these people are also used to estimate the

proportions eligible; thus, our CIs above are conservative.

6.10.b. Intervention Effect

We use the "intention to treat" approach assuming participants are grouped according to randomization for all analyses of the intervention effect. The stratified recruitment and randomization allow us to compare mean log(IL-6) between each of the three active groups to placebo using a linear mixed model with both main effects and their interaction, adjusted for baseline log(IL-6) and strata. Time is a repeated factor, and the primary comparison is based on a contrast at 12 months. The covariance structure will be assumed to be unstructured. The primary focus is on marginal comparisons (135 and 165/group) between each active intervention and placebo using one-sided hypothesis tests at the 10% level (we are not looking for definitive evidence, but to rule out small effects). We assume 5% loss to follow-up (LTFU); this is similar to the 5% we achieved in LIFE. Using the root mean squared error from LIFE-P (adjusted for baseline log(IL-6) and treatment) of 0.52, we have 91% power to detect a difference if the difference (on the log scale) is at least 0.1625. This is equivalent to the difference between 4.20 and 3.57 pg/ml (or a 15% difference). We would have 66% power for a 10% difference (4.20 vs. 3.78 pg/ml, 0.1054 on the log scale) and 99% power for a 20% difference (4.20 vs. 3.36 pg/ml, 0.2231 on the log scale). Individual treatment group comparisons (at least 60 vs. 75/group) would have 44% power for a 10% difference, 68% power for a 15% difference, and 87% power for a 20% difference. This achieves the first part of Aim a (i). As sensitivity analyses, we also explore the impact of additional factors (e.g., diet, physical activity, medication use) by adding these factors to the models. Although the mixed model is robust against data missing at random (MAR), multiple imputation will be used in sensitivity analyses.

From LIFE-Pilot, the SD of 400 m walk speed in IL-6 eligible participants is 0.21 m/s, with high correlation (r>0.80) between baseline and 12 month measures. With at least 60 and 75 participants per group (for pairwise comparisons), we project to have >99% power to detect a difference in means of >0.095 m/sec using a one-sided test at the 10% level (a substantial meaningful change).[192] There is also 67% power to detect a >0.038 m/sec difference (a small meaningful change). Using groups of 135 and 165 (for main effects), we would have >99% power to detect >0.095 m/sec, and 86% power for a difference >0.038 m/sec in walking speed. This analysis uses linear mixed models, as for IL-6; a similar approach is used for the secondary outcomes of SPPB, frailty, and grip strength. This achieves the last part of Aim a (i). For people who are unable to complete the 400m walk, we will use their walking speed from the portion completed.

6.10.c. Associations between inflammation and function

We base our calculation of detectable SPPB effects on LIFE data. The correlations between baseline and 24 month follow-up SPPB outcomes were 0.49 (Successful Aging: SA) and 0.47 (Physical Activity: PA); we assume a correlation of 0.5 between baseline and 12 month in ENRGISE. Estimated standard deviations at 24 months were 2.60 (SA) and 2.47 (PA); we assume 2.5. Under these assumptions, for the comparisons of 60 to 65 people, we have 90% power to detect a difference of at least 0.99 SPPB units; for the comparisons of 135 to 165 people, we have 90% power to detect a difference of at least 0.66 SPPB units. Both assume 1-sided tests at the 10% level.

We will examine the relationship between the change in IL-6 and the change in walking speed using univariate regression. Assuming we assess ≥285 participants (of 300, ≥95%) at 12 months, we have 80% power to detect a correlation less than -0.126 (i.e. at least 1.59% of explained variability) using a one-sided test at the 10% level. We have 90% power if the correlation is less than -0.152 (Aim a (iii)).

To assess intra-person variability in IL-6 (Aim a (iii)), we measure IL-6 at both screening visits. We use a Bland-Altman plot to assess agreement; this is summarized using a 95% CI of the difference at the two visits providing information on the expected differences over one week due to biological variability and measurement error. If agreement is high, one measure is likely sufficient for recruitment into the main study. If not, we may need to use two or more measures to determine eligibility and to assess participant-specific risk.

6.10.d. Additional inflammatory biomarkers:

In addition to IL-6 levels, we examine additional markers of inflammation (Aim b (i) and (ii)). As with IL-6, our primary focus is on the relationships between changes in cytokines with the change in walking speed using linear mixed models. We use stratified linear mixed models (as described above for IL-6) to examine the impact of interventions on these cytokines. These analyses assess whether changes in these cytokines are of similar magnitude to IL-6. As the absolute levels differ between cytokines, focus is on relative changes from baseline. We also examine these cytokines in models also including baseline and follow-up log(IL-6) to assess whether they provide additional predictive information. We also explore MMD event rates by baseline levels of IL-6 and additional cytokines to explore whether we can use an additional cytokine to select a population at greater risk to use for the main study, recognizing the potential tradeoff between ease of recruitment and selecting a population at higher risk.

6.10.e. Informing the main study

We assess compliance and retention rates (Aim a (ii)). Although we expect to be underpowered for incident mobility disability, we collect these data and compare estimates of event rates to those obtained in SA group LIFE participants that would have been eligible for ENRGISE (Aim a (ii)). While power is limited, we also examine whether there is sub-additivity and interaction of interventions in stratum 3, which supports the decision of whether to proceed with a factorial or parallel group design in Phase 3 (Aim a (ii)). .

We integrate all available information to make a determination about which approaches to carry forward into the main study. Weighed in this decision is recruitment yield (is it practical to consider a full size trial?), safety and tolerability (do participants tolerate the interventions?), changes in IL-6 in the pilot studies, the relationship of change in IL-6 with change in walking speed, the conclusions from our data analysis of other studies (e.g. LIFE, Look AHEAD, Health ABC, CLIP), and a revised literature search. Finally, we combine all information to estimate the sample size and **cost of the main trial** (Aim a (ii)). For example, for a two-arm study, we would need 2,084 people to have 90% power to detect a 20% (HR=0.8) reduction in MMD assuming a two-sided test at the 5% level, a control group event rate of 18.3%/year, LTFU of 5%/year, 18 months of recruitment, and a total study length of 4 years. To detect a 25% effect (HR=0.75), we would need 1,296 people. These calculations use the estimated MMD rate of 18.3%/year in the control group among people in LIFE-P whose baseline IL-6 was between 2.5 and 10 pg/ml.

7. Possible Discomforts and Risks

7.1. Confidentiality

Lack of maintenance of confidentiality is a potential risk. The information below relates to all collaborating performance sites for the proposed study. Data are used only in aggregate and no identifying characteristics of individuals will be published or presented. Results of testing are sent to participant's private physicians if participants agree to this. Alert values for medically relevant procedures (e.g., blood pressure, pulse rate, MMSE, blood tests) are sent to participants and participants' physicians, depending on the urgency of the values.

Confidentiality of data is maintained by using research identification numbers that uniquely identify each individual. Safeguards are established to ensure the security and privacy of participants' study records. Appropriate measures are taken to prevent unauthorized use of study information. The research ID number is used. The research records are kept in a locked room in the Field Center. The files matching participants' names and demographic information with research ID numbers are kept in a locked file. Only trained and certified study personnel has access to these files. After the study is completed, local data are stored with other completed research studies in a secured storage area.

In compliance with the Health Insurance Portability and Accountability Act (**HIPAA**) and the Standards for Privacy of Individually Identifiable Health Information of the Department of Health and Human Services, we access personal health information and medical records only after receiving signed informed consent.

7.2. Biological samples repository
We comply with the Office of Extramural Research requirements and guidelines related to the research use of stored biological specimens. The repository is subject to the oversight of the University of Vermont IRB which reviews and approves the protocol, training documents, and an informed consent document, according to the elements described above, for distribution to collector-investigators and their local IRBs.

7.3. Safety, risks, risk protection and dose adjustment related to drug administration is discussed above in section 6.

7.4. Safety measures during the assessments

All study assessments are done by staff trained and certified in Good Clinical Practice, study procedures and assessments, and safety procedures. The study nurse/medical safety officer reviews all health assessments, vital signs, medical history, medication use, and blood tests. All assessors receive **CPR training** and training on management of acute events including syncope, chest pain, acute dyspnea and abnormal vital signs. All sites have on-call access to contact numbers for emergency services.

Phlebotomy. The risks of drawing blood from a vein or finger stick include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, faintness from the procedure.

Blood pressure measurement. The risks of placing a blood pressure cuff on a participant's arms are that it may cause pinching or slight bruising.

400 m walk test. The 400 m walk test may be associated with the risk of falling or development of chest discomfort due to coronary ischemia or dyspnea due to heart failure or lung disease. Rarely, falling during the 400 m walk test may result in a fracture.

Research staff members that collect data are trained and certified in 400 m walk testing before they work with study participants. Study staff members are trained not to administer the 400 m walk test if they feel or the participant feels that testing is unsafe. Safety precautions are taken during the 400 m walk test by applying standardized stopping criteria. If the participant reports pain, tightness or pressure in the chest, significant shortness of breath, feeling faint, lightheaded or dizzy, or significant other medical problems the test is stopped. In addition, participants are asked whether they feel the test is safe. Those who state it may be unsafe are not allowed to complete the test. Staff members are trained to protect against falling and are trained in CPR. They are trained in activating the local emergency response system. In our experience conducting thousands of 400 m walk tests on individuals within various studies, the risk of falling is less than 1/500 and the risk of a fracture-associated fall is less than 1/5000.
In the LIFE study we have performed approximately 10,000 400 m walk tests with no safety concerns raised by the DSMB.

SPPB assessment. Similar to the **400 m** walk test, completion of the SPPB may be associated with the risk of falling or development of chest discomfort due to coronary ischemia or dyspnea due to heart failure or lung disease. Rarely, falling during the SPPB test may result in a fracture. Research staff members who collect data are trained and certified in SPPB measurement.

Strength measures, may rarely cause pain or muscle sprain. In our experience conducting thousands of measures, the testing has demonstrated to be safe.

Questionnaire administration.
Participation includes a risk of loss of confidentiality of personal health information. A number of methods are employed to maintain confidentiality of participants. First, questionnaire data are collected in secure spaces where the interview cannot be overheard. Secondly, only study investigators and key research staff (i.e. study

programmers and biostatisticians) have access to the study database. Third, participants are assigned a unique study identifier. Individual names are removed from the study database and only the unique study identifier is used to distinguish participants in the database. Fourth, collected data are maintained in locked computer files and file cabinets to which only study investigators have access. Collected data are used only for research purposes. Published data will not contain any individual identifiers.

Participant burden and fatigue

The duration of clinic visits for screening, baseline, and follow-up range between **15 min to 3 hours.** If a participant becomes too fatigued we make arrangements for a second visit or to complete the testing in the participant's home. Based on our experience with LIFE-Pilot, LIFE and other RCTs in the elderly (SHEP),[194] with observational studies in healthy older persons (CHS[195] and Health ABC[196]), and with disabled elderly women (WHAS),[197] visits lasting between 2 and 4 hours did not negatively affect annual follow-up **retention**, which was >90%**.** We closely **monitor** the potential impact of visit burden on retention. This information will be used to promptly implement strategies to promote retention and adherence.

7.5. Data Safety and Monitoring Plan

Monitoring adverse events

Safety of the study participants is always our major concern. The **Intervention and Safety Committee** provides early monitoring of adverse events and ensures standardization of clinical practice and safety issues across all sites.

An **adverse event** is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

The event is **serious** if it results in death, is life-threatening, requires inpatient hospitalization or prolongs an existing hospitalization, results in a persistent or significant disability/incapacity, or congenital anomaly/birth defect.

Safety related events are reported in a timely fashion, as required by the NIH, FDA, DSMB and the IRBs that are responsible for study oversight.

The classification of potential relationship to the intervention is as follows.

Definite	Temporal pattern + Known or expected AE response pattern + Confirmed by stopping the intervention + Reappearance of AE on re-challenge
Possible	Temporal pattern + Known or expected AE response pattern + Could have been produced by a number of other factors
Not related	AE for which sufficient information exists to indicate that the cause is unrelated to the study intervention

ENRGISE study participants are comprised of a frail, elderly population expected to be at high risk for acute and chronic comorbid health events.

A **Data and Safety Monitoring Plan (DSMP)** is implemented to ensure the safety of all participants involved in the study and to ensure the validity and integrity of the data. The Principal Investigators with the advice and assistance of the Intervention and Safety Committee described below and the Steering committee monitor all aspects of safety. The Intervention and Safety Committee reviews all Serious, Unexpected, and On-site adverse events and makes recommendations to the Steering Committee for any changes in reporting, consent or study activities.

A **Data Safety Monitoring Board (DSMB)** is established, with responsibility to monitor all aspects of the study, including those that require access to any masked data. The DSMB and its chair are named and approved by the NIA. It is planned that the DSMB meets by conference call as determined by the DSMB and the NIA. The DSMB has access to all study data, documents and progress. The Intervention and Safety Committee**, comprised of safety personnel from each site, the Chair, and a representative of the DMAQC** reports to the DSMB for issues related to participant safety.

The DSMB has the following charges:

- Review the study protocol.
- Review data (including masked data) over the course of the trial.
- Identify problems relating to safety over the course of the study.
- Identify needs for additional data relevant to safety issues and request these data from the study investigators.
- Propose appropriate analyses and periodically review developing data on safety and endpoints.
- Make recommendations regarding recruitment, treatment effects, retention, compliance, safety issues and continuation of the study.
- At any time, the DSMB may recommend discontinuation of any component/treatment group of the study

Finally, the NIA makes the final decision on whether or not to accept the DSMB's recommendation about discontinuation of any component of the study. Any serious adverse event that might be due to the study intervention are reported to the DSMB, the IRBs, and to the NIA Project Office. The exact timeline for reporting serious adverse events is determined by the DSMB, IRBs, and NIA.

8. Potential benefits of the proposed research to subjects and to others

There is direct benefit to the study participants, including study blood tests and a comprehensive assessment of physical function. All study participants are encouraged to communicate the results from the study to their primary care providers.

ENRGISE will provide critical information regarding the efficacy of the study interventions in averting low-grade chronic inflammation and mobility impairments in vulnerable older adults. The results of ENRGISE will have relevant clinical and public health implications, and will fill an important gap in knowledge for practicing evidence-based geriatric medicine.

We believe that the aforementioned risks of this study are minimal and reasonable, when compared to 1) the scientific knowledge to be gained by performing these studies as well as 2) the potential benefits to study subjects.

9. Conflict of Interest

None

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