

Version Date: 3/2/2018

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Study Title: Body Fat Reducing Effect and Safety of Enzymatically Modified Isoquercitrin in Overweight and Obese Subjects

I. Aim and Hypotheses

Specific Aim 1: To compare the body fat reducing effect of 360mg/day enzymatically modified isoquercitrin (EMIQ) with that of a placebo.

Hypothesis 1: Compared to a placebo, participants receiving EMIQ supplementation will have a greater reduction in total body fat as evidenced by measures of whole body DXA scan, skinfold thickness, waist to hip circumference ratio, resting metabolic rate, plasma adiponectin levels, physical activity, and dietary intake.

Specific Aim 2: To determine the safety of long term intake of EMIQ.

Hypothesis 2: Long-term intake of EMIQ has no ill effect on participant health as evidenced by measures of heart, liver, kidney function, and psychological state.

II. Background and Rationale

Dietary quercetin is a natural bioactive polyphenol with potential health benefits and the capacity to prevent development of various diseases including those of the cardiovascular system, central nervous system and others [1-3]. It is widely distributed in various plant foods such as onions, broccoli, concord grapes, apples and more [4]. In plants, quercetin is present as conjugated with different forms of glucosides [5], which affect their bioavailability. The bioavailability of different forms of quercetin has been the subject of several investigations [6]. Enzymatically-modified isoquercitrin (EMIQ) is a mixture of quercetin monoglucoside and its α -oligoglucosides, which mainly consist of quercetin-3-O- β -glucoside (Q3G) and α -glucosyl derivatives with 1-7 of additional linear glucose moieties [7, 8]. Supplementing the diet of apoE-deficient mice with EMIQ increased quercetin levels and suppressed atherosclerosis in this mouse model of human atherosclerosis[9]. In human trials, the bioavailability of EMIQ

has been shown to be 17-fold higher than quercetin aglycone [10]. After efficient absorption of EMIQ, quercetin is released, and it exerts its biological activity such as reducing body fat, improving cardiovascular health and reducing blood pressure.

EMIQ has been shown to be effective in reducing body fat in Japanese people who have different dietary habits and lifestyle from that of a person in the United States. Thus, this study is designed to investigate if EMIQ is also effective in reducing body fat in subjects with a western lifestyle.

Quercetin, according to Seo et al [11] in addition to being a very strong antioxidant, prevents adipogenesis by regulating two sets of enzymes, a) by upregulating adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) expression and, b) by downregulating fatty acid synthesis (FAS), lipoprotein lipase (LPL) and adipocyte fatty acid-binding protein (aP2) expression, through expression of transcription factors such as C/EBP α , PPAR γ , SREBP-1. These observations suggest that quercetin has therapeutic potential for body fat reduction through regulating the expression of transcriptional factors and enzymes associated with adipogenesis. Dong et al [12] also reported that dietary quercetin reduces high fat diet-induced body weight gain, fat deposition, and insulin resistance and suppresses glucose uptake by adipose tissue. They reported that these effects of dietary quercetin is mediated through quercetin activating of AMPK/ACC signaling pathway which promotes fatty acid β -oxidation and suppresses fatty acid esterification into triglyceride in adipocyte. Administration of 275 mg/d for 12 weeks reduced total body fat, visceral fat and subcutaneous fat in Japanese subjects. EMIQ is classified by the Food and Drug Administration (FDA) as Generally Recognized As Safe (**GRAS**). Thus, it should be considered a safe compound in order to investigate its potential biological activities in humans.

III. Research Plan

A. Experimental Design

This is a randomized, double-blind, placebo controlled study.

B. Sample Size and Statistical Analyses

50 subjects will be required to complete this 12 week study (though 150 will be screened and 64 will be enrolled to ensure completion of 50 subjects). Subjects will be randomized 1:1 into either the treatment (EMIQ) or placebo group. The study physician, Dr. Lisa Ceglia, will hold the code to the randomization which will be revealed upon completion of the study or if adverse event occurs.

To calculate number of subjects needed per group and provide 80% power at $\alpha=0.05$ we used mean differences and standard deviation (EMIQ: 19 ± 24.62 and Placebo: 1 ± 12.87) of whole abdominal fat loss from an early published study conducted in Japan, where the Japanese volunteers consumed 275mg EMIQ per day for 12 weeks and showed a positive impact of EMIQ on reduction of fat depots. We plugged in data from that study into this formula $N=1+[16(xS^2/d^2)]$ to calculate sample size. Using this formula the number of subjects in each group were calculated to be $n=18$. However, we selected to use 25 subjects per group which increases power of study to $>85\%$. 25 subjects per group is a conservative sample size, which make us to expect a larger difference in the means with a higher dose of EMIQ intake.

The primary objective of this study is to demonstrate reduction of total body fat following 12 weeks of EMIQ intervention. Therefore, we will determine statistically if EMIQ is effective to reduce percent body weight, total abdominal fat, subcutaneous and visceral fat after 12 weeks of intervention compared to baseline values. We plan to compare the two group medians with the Mann-Whitney U test. After reviewing the data at the completion of the study, we can assume normality, then we will compare the two group means with parametric t-test.

C. Subject Characteristics

Inclusion Criteria:

- Male or female age 18-65 years.
- Premenopausal female with regular menstrual cycle or post-menopausal female with cessation of menstrual cycle for a minimum of 6 months.
- BMI in the range of 26.0-37.0_
- Beck Depression Inventory Score less than 20.
- Fluency in spoken or written English.
- Willingness to be randomized to take EMIQ or placebo.
- Must weigh at least 164lbs

Exclusion Criteria:

- Any major illness or condition that may interfere with study outcomes at the discretion of the study physician.
- Diabetes Type I & Type II, or use of any pharmacological treatment for diabetes.
- Use of medications that interfere with energy metabolism
- Receiving hormone therapy growth hormone, testosterone, or estrogen with the exception of hormone contraceptives.
- GI diseases, conditions or medications known to influence GI absorption including active peptic ulcer disease or inflammatory bowel disease such as

- ulcerative colitis, Crohn's disease, celiac disease, chronic diarrhea or constipation.
- Undergone gastric bypass or other bariatric weight loss procedure.
 - Lipid lowering medications such as: bile acid sequestrants (Cholestyramine, Colestipol, Colesevelam, etc.), cholesterol absorption inhibitors (Ezetimibe-Zetia), and fibrates (Gemfibrozil, Clofibrate, Fenofibrate, Triclor). Exceptions to this exclusion are HMG-CoA reductase (Statins).
 - On or planning to participate in a weight loss program.
 - Weight loss or weight gain greater than 10 lbs in the past 6 months.
 - History of eating disorders, anorexia, bulimia, or binge-eating in the past 5 years.
 - Participation in any regular endurance exercise: running, biking, aerobics (except walking) or resistance training greater than 2.5 hours per week.
 - Regular use of acid lowering medications (greater than 3 times per week) such as antacids, PPIs (proton pump inhibitors), or H2 blockers.
 - Unstable thyroid disease.
 - Psychiatric disorders including schizophrenia, bipolar, major depression or psychosis.
 - Antidepressant medication: SSRIs (selective serotonin reuptake inhibitors), SNRIs (serotonin norepinephrine reuptake inhibitors), MAO inhibitors, or TCAs.
 - Cancer of any type (except for non-melanoma skin) in the past 5 years.
 - Use of chemotherapeutic agents.
 - Pregnancy, planning to become pregnant during the study period, or breastfeeding.
 - Unwilling to use effective birth control during study.
 - History of bilateral mastectomy with nodal dissection.
 - Alcohol use, on average, greater than 3 servings per day, 20 servings per week (Serving size: 12oz beer, 4oz wine, 2oz hard liquor), or self-reported binge drinking.
 - Uncontrolled hypertension (HTN) determined at the discretion of the study MD or RN. HTN medications allowed in the study: angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers, and diuretics.
 - Medications for chronic obstructive pulmonary disease (COPD) or kidney disease.
 - α -adrenergic or β -adrenergic blockers (oral or ocular) and diuretics.
 - Renal or chronic kidney disease due to any condition, renovascular disease, or dialysis.
 - Chronic liver disease such as hepatitis B, hepatitis C, or cirrhosis.
 - Cardiovascular disease including: myocardial infarction, cerebrovascular disease (CVA), coronary artery bypass graft, stenosis greater than 50%, angina, coronary artery disease (CAD), congestive heart failure (CHF), peripheral vascular disease (PVD) or dysautonomia.
 - History of autoimmune diseases such as rheumatoid arthritis, lupus, multiple sclerosis, vitiligo, or psoriasis.
 - HIV or AIDS based on self-report.
 - Steroid use with the exception of over-the-counter topical and nasal steroids such as Flonase.
 - Allergy medication or regular antihistamine use.

- Seizure disorders. Acceptable if managed with medication and free of seizure activity for 5 years.
 - Smoking or the use of nicotine replacement products in the past 6 months.
 - Use of dietary supplements containing vitamins (except Calcium and Vitamin D), minerals, herbal or plant-based preparations, fish oil or homeopathic remedies during study participation unless willing to discontinue prior to enrollment.
 - Current participation in another research study.
 - Non-English speaking.
 - No social security number.
 - Participation in another research study.
- **Withdrawal/Termination Criteria:** Low compliance including taking less than 80% of study supplements, or missing study visits. Subjects who manifest an allergic reaction to the study supplement or placebo. Negative health effects as determined by the study physician. In the event of a withdrawal/termination, study subjects will be asked to discontinue their study supplement, and, if necessary due to negative health effects, will be referred to their primary care physician.

*Note: Screening lab values that would interfere with study outcomes are determined at the discretion of the study physician.

D. Risk/Benefit Assessment: We do not anticipate any major risks or hazards to participants in this study.

There are no benefits to participation in this study. EMIQ has been independently evaluated by an expert panel as safe and has been categorized as GRAS (generally recognized as safe) according to the Food and Drug Administration (FDA)(see attachment “5. FDA GRAS EMIQ”). Other investigators have conducted similar clinical studies with EMIQ at doses comparable to those in this study and reported no adverse effect.

While all measures to ensure confidentiality will be taken, it is an ever present possibility that it may be lost. Stored samples will be labeled with a unique number (code) that will not contain any private information such as name or date of birth to ensure the samples cannot be identified. There is no intention for allowing anyone other than the principal investigator to have access to these codes. If Dr. Mohsen Meydani turns this study over to a new principal investigator, he will give the key code to the new principal investigator.

During blood draws there may be a small amount of bruising and discomfort, and rarely infection. Every effort will be made to minimize discomfort to volunteers. There may be discomfort associated with 12-hour fasting. Some subjects may experience dizziness during prolonged fasting.

There is minimal risk of psychological or cognitive dysfunction due to the intervention. Participants may experience claustrophobia during the DXA and RMR measurements. As such, a research technician will be present at all times during these procedures. If a volunteer indicates discomfort during the measurement, the procedure will be terminated at that time.

DXA scans expose participants to minimal radiation equivalent to 0.73 days total of background or natural radiation. This is equivalent to the amount of radiation exposure experienced by spending about 18 hours outdoors in a given day.

E. Subject Participation

I. Overview

This study will be a double-blind and placebo-controlled study. Total of 50 overweight and obese subjects with BMI: 26-37 will be randomly assigned into two groups: 25 subjects to placebo control group and 25 subjects into treatment group. Subjects in both groups will be asked to consume their regular diet during the 12 weeks of the study period. Subjects in the treatment group will also be asked to swallow two capsules of 180mg each, totaling 360mg of EMIQ (enzymatically-modified isoquercitrin) every day, one capsule at breakfast and one at dinner, for a period of 12 weeks. The subjects in the placebo group will also consume their regular diet and will swallow two identical placebo capsules, one at breakfast and one at dinner, for 12 weeks.

According to San-Ei Gen, the producer of EMIQ, the No Observed Effect Level (NOAEL) of EMIQ is 489 mg/kg BW/day, and the Acceptable Daily Intake (ADI) is 4.89mg/kg BW/day [13, 14]. This data was generated from several studies including: acute oral toxicity testing [15], 4 week oral toxicity testing [16], 13 week oral toxicity testing [17-19], mutagenicity testing [20], and 2 year carcinogenic testing [13].

The goal of this study is to test the effect of EMIQ on body fat reduction in overweight and obese individuals. Thus we have selected a BMI range of 26-37 kg/m², assuming an average height of 1.72m (5' 8") and an average weight of 94kg (207.2 lbs).

To calculate the dose of 360 mg EMIQ/day that participants will receive, we used the dose administered in the previous human studies from Japan as a benchmark. We wanted the dose in our study to reflect the greater average predicted weight of our participants compared to a person in the Japanese study due to an increase in BMI range for our study. The BMI range for the study completed in Japan was 24-31, while the range for this study is 26-37. Japanese study subjects weighed approximately 72kg while our prediction is that participants will average 94kg based on our database records. This % difference (31%) is reflected in the increase from 275mg/day to 360mg/day.

Our rationale for setting a minimum weight requirement is that a subject may be within the BMI range outlined in the study, but still not weigh enough to meet the ADI of 4.89mg/kg BW/day. A minimum bodyweight of 74kg should place participants in a safe range below the specified ADI. We believe that a minimum body weight of 74kg should be a requirement for study participation. This rationale is demonstrated in the following calculation:

$$\begin{aligned}4.89\text{mg/day} \times (\text{x})\text{kg} &= 360\text{mg} \\ (\text{x})\text{kg} &= 360/4.89 \\ (\text{x})\text{kg} &= 73.62 \text{ (162.3lbs)}\end{aligned}$$

This study will be carried out after review and approval of the Institutional Review Board (IRB) of Tufts University. Following IRB approval, a total of 50 male and female volunteers will be recruited and admitted to the study. The scope and plan of the study will be described to the admitted volunteers and a written consent will be obtained before enrolling the subjects into the study. The study is planned to start in 2016 (i.e., begin enrollment) and volunteer activity completed within one to one and a half years.

The admitted volunteers will be scheduled and asked to come to the Metabolic Research Unit (MRU) at Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University at the beginning of the study for enrollment (visit 1), baseline (visit 2), 4 weeks after baseline (visit 3), a visit 8 weeks after baseline (visit 4), and the final visit after 12 weeks (visit 5) for a total of 5 visits. Overnight fasted blood samples will be collected at screening and visits 2, 3, 4 and 5.

In order to monitor adherence and safety of EMIQ intake over the 12 week of the study, volunteers will undergo electrocardiogram (EKG) testing as well as several hematological tests, liver functions tests and other biochemical tests (See below) carried out on the collected blood samples. In addition, volunteers will be asked to provide a spot urine sample during screening and visits 2 and 5 to measure total protein, glucose, urobilinogen, bilirubin, ketones, pH and occult blood in urine using urine dip stick test. Volunteers will complete three (3) 24-hour dietary recalls over the phone at three time points: between visit 1 and visit 2; between visit 3 and visit 4 (around week 6 from beginning the supplement); and between visit 4 and visit 5 (around weeks 11-12) to estimate total caloric intake and intake of three major nutrient groups: protein, carbohydrates, and fat. Volunteers will be asked to bring back unused EMIQ capsules and a compliance calendar during each visit to determine adherence to the supplementation schedule. Volunteers will be asked not to change their regular physical activity, and will fill out a 7-day physical activity record at visits 2, 3, 4 and 5 to monitor this. To assess changes in adiposity following EMIQ intervention, the volunteers will complete whole body and segmental Dual X-ray Absorptiometry (DXA), skinfold thickness and hip to waist circumferences measurements at visit 2, i.e., before the intervention, and after 12 weeks of intervention, at visit 5, for total and segmental changes in body fat and fat free

mass. DXA data will be further subjected to computational analysis to obtain changes in abdominal, visceral and subcutaneous fats.

Per policy number 9.20.18.30 of Tufts Department of Pharmacy Policy and Procedure Manual, any supplement returned from study subjects, or leftover upon completion of the study, will be destroyed “on-site” by placing them in a chemotherapy waste container for disposal using the Hospital’s system.

The following is a summary of the 6 total visits required by volunteers in this study:

Screening: Volunteers that were pre-screened by telephone will be scheduled and asked to come to the MRU at the HNRCA. Volunteers will be asked to arrive fasted for 12 hours. When they arrive, volunteers will be given a Screening Consent Form along with an explanation of all procedures that will be completed at screening. Volunteers will have an opportunity to read the consent form and have any of their questions answered to their satisfaction. Once their consent is given we will begin by measuring volunteer’s vital signs, height, body weight wearing a pre-weighted pair of scrubs, and BMI. Blood samples will be collected for blood chemistry analysis. Women of childbearing potential will be subjected to a blood pregnancy test. The Beck Depression Inventory will be administered to assess for depression. Additionally, study inclusion and exclusion criteria and health history will be reviewed. Breakfast and a \$25 screening stipend will be provided once screening procedures are complete. All subjects will be mailed a copy of screening labs within 1-2 weeks following the screening visit. Volunteer’s enrollment eligibility will be determined based on meeting inclusion and exclusion criteria. Subjects with abnormal hematologic parameters, as defined by the HNRCA Nutrition Evaluation Laboratory (NEL) reference ranges, which are determined by the study physician to interfere with study outcomes, will be excluded from study participation. At the discretion of the study physician, subjects will be alerted to abnormal results that warrant follow-up with their primary care physician. Subjects with abnormal hematologic values outside the NEL reference range that do not affect study outcomes will be included at the discretion of the study physician. Note that data generated from blood draws at this visit will be used as baseline values in lieu of drawing additional blood at visit 2 for subjects that continue the study after screening.

Enrollment, Visit 1: Volunteers that meet the study inclusion and exclusion criteria will be invited to participate in the main study. Those interested will be asked to return to the HNRCA. Volunteers will be given a Main Study Consent Form as well as an Optional Tissue Banking Consent Form. Volunteers will have an opportunity to read the consent forms and have any questions answered to their satisfaction. Once consent is obtained, MRU staff will assess any volunteer health, medication or eligibility status changes. The Dietary Assessment Unit (DAU) will dispense instructions for completing 24-hour dietary recalls by phone and will complete 3 dietary recalls by phone between visit 1 and visit 2. A baseline visit will be scheduled. Subjects will be mailed a \$50 check for completion of Visit 1.

Baseline, Visit 2: Subjects will be asked to arrive at the MRU fasted for 12 hours. Vital signs, and EKG measure of heart rhythm, scrub weight and height will be obtained. Changes to volunteer health, medication and eligibility status will be assessed. Blood samples will be collected and archived pending tissue banking consent. Several baseline measurements will be performed during this visit including: lipid profile, insulin test, adiponectin, a urine dip stick, skinfold thickness and waist/hip circumference, resting metabolic rate (RMR), and DXA measure of total body fat. To perform RMR analysis, subjects will be asked to rest for 30 min prior to the test conducted by study staff. Systolic and diastolic blood pressure and RMR will be measured after 30 min rest. Subjects will be escorted to The Body Composition Analysis Center (BCAC) of the Tufts University Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy for a whole body DXA scan to measure total and segmental body fats. A three factor eating questionnaire, food craving questionnaire, and the Stanford 7-Day Physical Activity Recall (PAR) will be administered to assess volunteer's dietary inhibition, restraint, hunger, cravings and physical activity level. EMIQ capsules, compliance calendar, and instructions will be dispensed to participants by nursing staff in the form of 180mg capsules contained in blister packages at the quantity to last for 1½ months. A continental breakfast meal will be provided to the subjects. Subjects will be mailed a \$150 check for completion of Visit 2. Subjects will be instructed by MRU staff to store the study supplement at room temperature and away from heat and moisture. These instructions will also be attached to the study supplement in the form of labels.

Week 4, Visit 3: Subjects will be asked to return to the MRU 4 weeks (± 1 week) from visit 2. This visit is required to monitor volunteer health and safety during EMIQ supplementation of 360mg/day for 4 weeks. Subjects will be asked to arrive fasted for 12 h and bring their unused supplements as well their compliance calendar. Vital signs, scrub weight and changes to volunteer health, medication and eligibility status will be assessed. Blood samples will be collected for liver and kidney function. In addition, a second EKG will be performed to monitor any changes the supplement may have on heart function. The PAR will be administered. Compliance with supplement intake will be determined by counting the unused capsules remaining in blister pack and review of compliance calendars. The second 1 to 1½ month supply of EMIQ capsules will be dispensed to the subject. A continental breakfast meal will be provided to the subjects. Subjects will be mailed a \$50 check for completion of Visit 3. After visit 3 (around week 6), subjects will complete 3 dietary recalls by phone.

Week 8, Visit 4: Subjects will be asked to come to the MRU 8 weeks after visit 2 or 4 weeks (± 1 week) after their previous visit. This visit is also required to monitor and ensure safety of EMIQ supplement intake at the dose of 360 mg/day for 8 weeks. Subjects will be asked to arrive fasted for 12 h and bring their unused supplements as well their compliance calendar. Vital signs, scrub weight and changes to volunteer health, medication and eligibility status will be assessed. Blood samples will be collected to monitor liver and kidney function. The PAR will be administered. Compliance with supplement intake will be

determined by counting the unused capsules remaining in blister pack and reviewing the compliance calendar. The final 1 to 1½ month supply of EMIQ capsules will be dispensed to subjects. A continental breakfast meal will be provided to the subjects. Subjects will be mailed a \$50 check for completion of Visit 4. After visit 4 (around week 11-12), subjects will complete 3 dietary recalls by phone.

Week 12, Visit 5: Subjects will be asked to return to the MRU approximately 12 weeks from their baseline visit or 4 weeks (\pm 1 week) from their previous visit. Subjects will be asked to arrive fasted for 12 hours and to bring back their unused supplement and compliance calendar. Vital signs, scrub weight and changes to volunteer health, medication and eligibility status will be assessed. Blood samples will be collected and archived pending tissue banking consent. Final measurements will be taken during this visit including: routine health screening analysis, lipid profile, adiponectin, insulin test, a urine dip stick, liver and kidney function (SGOT, SGPT, BUN, creatinine, and calculated GFR), skin fold thickness and waist/hip circumference. Subjects will be asked to rest for 30 min prior to measuring systolic and diastolic blood pressures and RMR analysis, which will be conducted by study staff. After RMR and BP measurements, subjects will be escorted to the BCAC for a whole body DXA scan to measure total and segmental body fats. A three factor eating questionnaire, food craving questionnaire, and the PAR will be administered to assess changes in volunteer's dietary inhibition, restraint, hunger, cravings and activity level throughout the study. Compliance with supplement intake will be determined by counting the unused capsules remaining in blister pack and by reviewing the compliance calendar. A continental breakfast meal will be provided to the subjects. Subjects will be mailed a \$305 check for completion of the final study Visit 5.

Below is an outline of the visits mentioned above:

Visit	Procedures	Length
Screening	Obtain Vital Signs Height, Scrub Weight and BMI 10mL Blood Draw* Urine Sample Review Inclusion/ Exclusion Criteria Review Health History Complete Beck Depression Inventory *Some women may be required to give an additional 3mL blood for a pregnancy test	2 Hours
Visit 1	Assess Health/Medication/ Eligibility Changes Receive Instructions/Materials for Dietary Recalls	1 Hour
Between Visits 1 and 2	No visit required. Volunteers will be called 3x for 24-hour dietary recalls.	
Visit 2	Assess Health/Medication/ Eligibility Changes Obtain Vital Signs Height, Scrub Weight and BMI Undergo EKG Measurement Urine Sample 30 Minutes Bed Rest Followed By RMR Measurement Hip/Waist Circumference Skinfold Thickness 17mL Blood Draw DXA Measurement Administer Questionnaires Receive Supplement/ Instructions/ Compliance Calendar	5 Hours
Visit 3	Assess Health/Medication/ Eligibility Changes Obtain Vital Signs Scrub Weight Undergo EKG Measurement 5mL Blood Draw Physical Activity Recall Questionnaire Return Unused Supplement/ Compliance Calendar Receive Next Round Of Supplement	2 Hours
Between Visits 3 and 4	No visit required. Volunteers will be called 3x for 24-hour dietary recalls.	
Visit 4	Assess Health/Medication/ Eligibility Changes Obtain Vital Signs Scrub Weight 5mL Blood Draw Physical Activity Recall Questionnaire Return Unused Supplement/ Compliance Calendar Receive Next Round Of Supplement	2 Hours
Between Visits 4 and 5	No visit required. Volunteers will be called 3x for 24-hour dietary recalls.	
Visit 5	Assess Health/Medication/ Eligibility Changes Obtain Vital Signs Scrub Weight Urine Sample 30 Minutes Bed Rest Followed By RMR Measurement Hip/Waist Circumference Skinfold Thickness 20mL Blood Draw DXA Measurement Administer Questionnaires Complete Beck Depression Inventory Return Unused Supplement/ Compliance Calendar	5 Hours

II. Registration

This study will be registered on <http://www.ClinicalTrials.gov>.

III. Recruitment, Screening and Consent

Participants will be recruited from the general public using several recruitment strategies including local print and electronic media (such as advertisements in newspapers, craigslist, local newsletters, bulletin boards and different websites, [i.e., university jobsites at Tufts University, Boston University]), social media (i.e. Facebook, Twitter, LinkedIn) as well as posting fliers in public places, with permission, such as local YMCAs, supermarkets, libraries, laundromats, local community organizations and health centers. Additionally, the Metabolic Research Unit (MRU) resources include a roster of >20,000 names of subjects from which potentially qualifying women and men can be identified and contacted through the use of direct mailings. Only subjects in the MRU database who have agreed to be contacted for future studies will be contacted for this study. Subjects who do not wish to be contacted are flagged in this database (Protocol Manager) with "DO NOT CONTACT." Previous subjects with this flag in their record will not receive any unsolicited recruitment materials, i.e., the letter to previous volunteers. All advertisements, fliers, and letters will be pre-approved by the IRB.

Although employees will not be targeted for recruitment and enrollment, employees of Tufts University and/or the HNRCA (employee-subjects) who voluntarily want to participate in the study will be eligible for screening and enrollment. In order to qualify for the study, employee-subjects must respond to IRB-approval advertisement of their own accord and will not be directly approached by any person seeking to recruit them for participation in the study. Members of the research team, as direct-report subordinates of the PI and anyone who is direct-report subordinate to any of the research team members in any other capacity will not be eligible to participate in the study. If employee-subjects qualify to participate in the study, they cannot participate as volunteers during hours in which they are being compensated by Tufts University for their regular work. This includes use of vacation, personal days and sick time.

Interested subjects will be pre-screened by telephone. Potentially eligible subjects will be invited to screen in-person for the study in order to further determine eligibility. During the screening visit, informed consent will be obtained by authorized MRU personnel using the screening informed consent form. The consent interview will take place in a private area in the MRU at the HNRCA. Once all of the participant's questions and concerns have been addressed, the

designated staff will obtain informed consent. Subjects will then undergo routine blood and urine tests. If subjects meet study criteria, they will be invited to participate in the main study. Results of the blood and urine testing will be available at least 1-2 weeks after screening. Subjects with lab values that do not meet eligibility requirements, assessed by the study physician or nurse, will be disqualified and may be provided with a recommendation to see their physician. Prior to study enrollment, subjects will be allowed to read the main study consent form. Once all of their questions and concerns are addressed, and they understand all procedures in the study, a designated staff member will obtain their informed consent using the main study informed consent form.

*Note: Non-English speaking individuals will not be enrolled in this study. The reasons being include the following: The HNRCA is not a health care facility, and does not have appropriate interpretive services to include non-English speakers. The standardized questionnaires have not been validated in other languages. This study will not directly benefit participants.

IV. Study Assessments

Blood and Spot Urine Collection: The MRU will collect a total of 60mL (12.2 teaspoons) of blood and three separate urine samples from each subject to measure the following:

Hematological measures: Complete blood count with auto-differential, white blood cell (WBC), red blood cell (RBC), hemoglobin (Hgb), hematocrit (Hct), platelet (Plt), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). WBC/Differential includes: neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Lipid profile: Triglycerides, Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol.

Metabolic and hormonal markers: Insulin, adiponectin in serum, and urine stick test (Includes: total protein, glucose, urobilinogen, bilirubin, ketone, pH, occult blood).

Liver function: Aspartate aminotransferase (AST), alanin aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase (GTP), total bilirubin (T-bill), lactate dehydrogenase (LDH), total protein (TP), and Albumin (Alb).

Muscle Function: Blood urea nitrogen (BUN), and creatine (Cre).

Electrolytes: sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg).

Blood and Urine will be collected according to the following scheme:

Screening: 13mL (2.6 teaspoons) of blood will be drawn at this visit. 5mL (1 teaspoon) will be drawn for complete blood count (CBC) with differential, and another 5mL (1 teaspoon) for routine blood chemistries. CBC measures will be used as baseline values. Additionally, participants in the screening process will be required to provide a spot urine sample for urinalysis. A 3mL (0.6 teaspoons) blood sample will be taken from women of childbearing potential at enrollment in order to conduct hCG testing. This test will determine whether the participant is pregnant, and thus ineligible for participation in this study.

Main Study:

Visit 1, Enrollment: No blood will be drawn during this visit.

Visit 2, Baseline: A 10mL (2 teaspoons) blood sample will be drawn into red marble top tubes for liver and kidney function tests, insulin and adiponectin. An additional 7mL (1.4 teaspoons) of blood will be drawn into lavender top tubes for analysis of lipids. A spot urine sample will be provided for urinalysis.

Visit 3, Week 4: A 5mL (1 teaspoon) blood sample will be drawn into a gold topped tube for liver/kidney function testing.

Visit 4, Week 8: A 5mL (1 teaspoon) blood sample will be drawn into a gold topped tube for liver/kidney function testing.

Visit 5, Final: A 3mL (0.6 teaspoons) blood sample will be drawn into a lavender topped tube for CBC differential analysis. A 10mL (2 teaspoons) blood sample will be drawn into red marble top tubes for liver and kidney function tests, insulin and adiponectin. An additional 7mL (1.4 teaspoons) of blood will be drawn into lavender top tubes for analysis of lipids. A spot urine sample will be provided for urinalysis.

*Note: Visits 3 and 4 will be used to monitor participant's liver and kidney function during the study. If the study physician determines that liver function appears to be in jeopardy, subjects will be asked to discontinue the supplement and to take medical action at the discretion of the study physician.

*Note: Abnormal lab values found at screening will be provided to the volunteers in writing. Abnormal values for safety labs (those falling outside of the study physician's predetermined range) will be repeated. The study physician will be notified and determine any follow-up action.

Nutritional Intake and Physical Activity:

Subjects will receive a Food Amount Booklet and information about the 24-hour recalls at visit 1. The Dietary Assessment Unit will collect three (3) 24-hour recalls by phone (two weekdays and a weekend) between visits 1 and 2. Three recalls will be collected by phone again between visits 3 and 4 (around week 6, midway through the 12 week supplementation period) and between visits 4 and 5 (around weeks 11-12). Dietary intake data will be analyzed using Nutrition Data System for Research software version 2015, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN.

Volunteers will be asked **not to change** their regular physical activity; unless, their total physical activity per week amounts to greater than 150 minutes, in which case they will be asked to refrain from exercise 30 days prior to participating in the study and to continue refraining from exercise until the end of the study. In addition, volunteers will be subjected to four PAR questionnaires to assess physical activity at visits 2, 3, 4 and 5. Subjects showing drastic changes in physical activity will be consulted, monitored, and if necessary, will be eliminated from the study.

To assess changes in dietary restraint, disinhibition and hunger, the Three Factor Eating questionnaire will be administered at both visits 2 and 5. In addition to this, general susceptibility to food cravings will be assessed using the Food Cravings Questionnaire trait administered visits 2 and 5.

Analysis of Whole Body Fat by DXA Scan:

To obtain whole body and segmental changes in body fat and fat free mass including trunk, arms and legs, as well as visceral and subcutaneous fat, two (2) DXA scans will be performed, one at visit 2 and another one at visit 5.

Fasting Resting Metabolic Rate/Respiratory Quotient:

Fasting RMR/RQ will be performed by a member of the study team at visits 2 and 5 to determine fat burning rate.

Electrocardiogram (EKG):

To ensure the safety and suitability of study participants, as well as of the supplement, two separate EKGs will be performed at visits 2 and 3. EKGs will be performed to assess heart activity and any changes that may occur throughout the study.

Vital signs and anthropometric measures:

The following measurements will be completed at selected study visits:

Body weight: screening, visits 2, 3, 4 and 5

Body height: screening, visits 2, 3, 4 and 5

Waist and hip circumferences: visits 2 and 5

Skinfold thickness: visits 2 and 5

Systolic blood pressure: screening, visits 2, 3, 4 and 5

Diastolic blood pressure: screening, visits 2, 3, 4 and 5

Heart rate: screening, visits 2, 3, 4 and 5

Subjects with weight loss of more than 10 lbs between visits will be evaluated by the study MD, and further action will be determined at his discretion.

Compliance:

Study participants will be contacted by a member of the study team each week either by phone or by email to monitor adherence to the intervention and to monitor self-reported health changes. Study team members will fill out a weekly log to ensure that volunteers have been contacted. If any abnormalities are reported, the study physician will be informed. The study physician will advise the participant to follow up with their primary care physician and also decide if the participant can continue with the intervention.

Participants will receive a compliance calendar used to track their supplement intake at visit 2.

Participants will be required to return any unused supplements and compliance calendar at visits 3, 4 and 5. Participants that miss a visit beyond the allowed interval (4 weeks \pm 1 week from previous visit) will be instructed to discontinue their supplement, if they have not done so already, and they will be removed from the study.

V. Location

This study will be performed at the Metabolic Research Unit and Vascular Biology Laboratory at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, located at 711 Washington St, Boston, MA 02111.

DXA scans will be performed at the Body Composition Analysis Center at Tufts University Friedman School of Nutrition and Science Policy, located at 150 Harrison Ave, Boston, MA 02111.

VI. Transportation

Volunteers will be required to provide their own transportation to the HNRCA. The building is accessible by public transportation. However, if

participants should choose to drive, they can obtain parking validation at a reduced cost.

VII. Personnel who will conduct the study

a. Present During Study Procedures:

Study Nurse
Study Coordinators
Principal Investigator

b. Primary Responsibility for the Following Activities

i. Obtaining Informed Consent

Authorized MRU staff
Principal Investigator
Study Coordinators

ii. Providing On-Going Information to the Study Sponsor and the IRB

Principal Investigator
Co-Investigator
Study Physician

iii. Maintaining Participant's Research Records

Authorized MRU staff
Principal Investigator
Study Coordinators

VIII. Subject Payment

Screening Stipend: \$25

Visit 1 Stipend: \$50

Visit 2 Stipend: \$150

Visit 3 Stipend: \$50

Visit 4 Stipend: \$50

Visit 5 Stipend: \$305

Subjects will only be paid for each completed visit, and will not be paid if they withdraw or are withdrawn from the study.

IX. Confidentiality

The identity of the volunteers, medical records and data relating to this research will be kept confidential, except as required by law and except for inspections by the USDA or the Tufts Medical Center

Institutional Review Board. The medical records will be filed in the medical record room only available to designated research staff. Upon entry into the research study, the volunteer will be assigned a unique code number. For storage, blood and urine samples will be identified with this unique code number (not related to medical record number), date, laboratory generated login number and sample description (i.e. plasma, serum, urine).

All samples and data generated from the research study will be labeled and/or recorded with a unique code number and no other identifier. The information that will allow the unique code number to be linked to the volunteer's name will be stored as a password-protected computer file. Samples will be stored until all analyses are complete. If blood samples collected during the course of this research study are sent outside the HNRCA for analysis, they will be labeled in a manner that contains no information that can make it possible to trace the sample back to the volunteer as an individual. At the conclusion of the study, the information file that would allow linking of the volunteer's identification information with the sample labels will be destroyed. After the research study is completed data and samples will be stored in a manner such that the identity will not be traceable.

Volunteers' records will be filed in the Metabolic Research Unit's Medical Records File Room located on the 11th Floor of the Human Nutrition Research Center. Research records, including volunteer records, will be retained for a period of 7 years after completion of the study.

Dr. Mohsen Meydani will have access to the key to identity codes. Dr. Meydani will have access to the de-identified data. Laboratory personnel responsible for analyzing the samples will have access only to the de-identified data. The study statistician will have access only to the de-identified data.

The study physician and nurses, the PI and Co-I, and Research Coordinators will have access to the research records.

X. Alternatives

Participation in this study is entirely the participant's choice. Participants may withdraw from the study at any time for any reason. Versions of the study supplement are available for purchase from vendors outside of the study.

XI. Payment

All stipends will be mailed to participants in the form of checks 2-3 weeks after they complete each visit. Participants who are removed from the study or choose to drop out will be paid for their completed visits only, and will not be paid if they are removed or choose to drop out before the completion of any study visit. In the event of a government shutdown or any other emergency situation, stipend will be delayed until the shutdown or emergency has ended.

F. Specific Procedures Used Throughout this Study

Distribution and Storage of Dietary Supplement

Supplements and placebo will be labeled in a binary (e.g., A or B) fashion without study staff's knowledge of which is which. Both supplement and placebo will be stored in locked cabinets at the MRU. The two will be distributed to participants, in accordance with their randomization scheme, starting at visit 2. Participants will receive an extra week's worth of supplements at each visit wherein supplement is distributed in anticipation of events that may keep volunteers from attending their next visit after 4 weeks.

Weight and Height:

Body weight will be measured during each visit to the HNRCA, aside from visit 1. Participants will be asked to change into scrubs provided by MRU during weight measurement to ensure the most accurate measurement.

Height will be measured at each visit to the HNRCA except enrollment. Shoes and head dressings or ornaments will be removed. Measurements will be taken according to standard procedure.

Waist and Hip Circumference:

Waist and hip circumference will be measured at visits 2 and 5. Measurements will be taken against the skin. Waist circumference will be measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Hip circumference will be measured around the widest portion of the buttocks with the tape parallel to the floor. These measures will be performed in duplicate and averaged.

Resting Metabolic Rate (RMR):

RMR measures will be performed only at the visits 2 and 5. Subjects will arrive at the HNRCA in a fasted state. Subjects will undergo bed rest for 30 min prior to measurement. Systolic and diastolic blood pressure will be measured. Measures of VO_2 and VCO_2 will be made

using an indirect calorimeter calibrated using test gases of known concentration. Subjects will be instructed to relax and avoid fidgeting and sleeping during the measurements. Measures will be made under thermo-neutral conditions for 40 minutes. Rates of energy expenditure will be calculated from VO_2 and VCO_2 using Weir's equation. Fasting oral temperature will be measured on days in which RMR is measured.

Dual-Energy X-Ray Absorptiometry (DXA):

DXA measurement will be performed only at the visits 2 and 5. Once subjects have completed measures at the HNRCA, they will be escorted to the Body Composition Analysis Center. The subject should be asked about and examined for metal that could be in the scan path. Typical items to look for are earrings, eyeglasses, wristwatches, coins, rings, buttons, buckles, zippers, and support braces. The subject must remove shoes, and it may be necessary to remove skirts, slacks, brassieres, etc. If clothes are removed, a medical cotton gown will be provided during the scan. Subjects will be asked to lie down on the scan table without allowing their hands and legs to overlap while the scan is performed. The analysis of each whole body scan will generate values for total body lean, total BMC, total body fat, total body mass, trunk fat, trunk lean, total bone mineral density (BMD), lumbar BMD, upper extremity fat, upper extremity lean, lower extremity fat and lower extremity lean. To minimize error, each patient will be scanned on the same machine from baseline to final. Scans will be analyzed by the Body Composition Analysis Lab.

Electrocardiogram (EKG):

An EKG will be performed by MRU staff at visits 2 and 3. Areas of participant's chest, arms and legs will be rubbed with alcohol and 12 electrodes will be attached to the skin. Participants will be required to lie still on their backs in bed while electrical activity is recorded by these electrodes. Electrodes will be removed after the test, which takes approximately 20 minutes to administer.

Skinfold Thickness:

To obtain more specific subcutaneous fat measurements we will perform skinfold thickness tests. Tests will be performed at visit 2 and visit 5. Participants will be asked to remove their shirts in order to obtain the measurements. Skinfold thickness will be measured at four sites: subscapular, suprailliac, the biceps and the triceps. All measures will be performed on the participant's right side unless it is not possible to do so. Sites will be identified through anatomical landmarks and measurements. The skinfold site will be marked. The participants skin will be pinched approximately 1cm above the mark using the thumb and index finger, and simultaneously pulled away from the underlying muscle to form the skinfold. While the pinch is maintained the caliper

jaws will be opened and positioned on the mark approximately 1cm below the thumb and index finger. The caliper jaws will be allowed to close around the skinfold. Two to four seconds after the caliper pressure is released the reading will be taken. This process will be repeated until subsequent measurements are within 1-2mm of each other. The subscapular measure will be taken from the bottom point of the shoulder blade. Suprailiac measure will be taken just above the top of the hip bone. Triceps will be measured at the posterior midline of the upper arm, and biceps at the anterior midline of the upper arm.

Questionnaires:

The following questionnaires will be administered:

Beck Depression Inventory: this questionnaire will be administered by MRU staff at screening and visit 5 to assess volunteer's psychological state. If subjects score ≥ 20 , they will be informed of the result and a recommendation will be made to follow up with their physician.

Three Factor Eating Questionnaire: This questionnaire will be administered by study team staff at visits 2 and 5.

Food Cravings Questionnaire-trait: This questionnaire will be administered by study team staff at visits 2 and 5.

Stanford 7-Day Physical Activity Recall: This questionnaire will be administered by study team staff at visits 2, 3, 4 and 5.

Volunteer Consent and Safety:

The protocol will be reviewed and approved by the Tufts Institutional Review Board (IRB), and written informed consent will be obtained from all participants. Study volunteers will be given the opportunity to read consent forms in a quiet area free of noise and distraction. Consent forms will be thoroughly reviewed with the volunteer and all of their questions will be answered to the volunteer's satisfaction. Comprehension of study information will be assessed by asking volunteers to restate their understanding of presented information in their own words. Participants will be told they can decline participation at any time for any reason. Participant identifiers will be kept in a separate file from data. Data collection forms will not have participant identifiers. The participant's identity and records relating to the prescreening and screening procedures will be kept confidential in locked cabinets at all times at the Volunteer Recruitment Services, and at the MRU, respectively, whether or not the participant qualifies for the study. The information kept may be used to invite the individual to participate in future studies at the HNRCA. If a participant chooses not

to have their contact or other information kept in our records for this or other reasons, the participant need only to contact the Volunteer Recruitment Services Department by telephone or email to make this request, and all of their records will be destroyed. All study records for all participants will be kept confidential, and only the study P.I., Co-P.I., study physician, and study coordinators will have access to participant identifying information, if needed; otherwise, only de-identified data will be transferred to co-investigators. All data will be kept in secure files and locked data cabinets. Identifiers will not be destroyed at any point.

Participants and nursing staff will be able to reach the study physician, Dr. Lisa Ceglia, or a covering physician 24 hours/day. Specific participant complaints will be addressed on an individual basis and appropriate referrals to primary care providers or other health care providers will be made. Participants' health status will be assessed weekly through phone or email and will be updated by the members of the study team. While there is no evidence to suggest so, there might be some adverse effects from EMIQ's effect on fat loss, or EMIQ itself. If these problems are reported by participants, the condition will be assessed to determine if these adverse events are potentially study-related. Further, in the case of these and any other adverse event, the study physician will advise participants on the appropriate assistance or action to take, and then counseled to seek further appropriate assistance.

Compensation will not be available for any research-related injury, and treatment may incur charges for the subject. The Nursing staff at the MRU is basic BLS (CPR) certified and trained in the use of an automatic external defibrillator (AED). In all emergency situations, the MRU Nursing staff will provide immediate basic life support (BLS) response, call 911 and continue to provide emergency supportive care as needed (e.g., monitoring, oxygen, airway maintenance) until emergency medical service (EMS) arrives. Their philosophy is to provide early access to the most appropriate and advanced care for treatment of study subjects. Treatment by the MRU Nursing staff and/or the staff physician is provided at no cost to the participants. All additional treatment including the cost of an ambulance is charged to the study subject. There are no funds available for these costs to be paid by the HNRCA or by study funds. This information is provided to participants prior to their signing an informed consent form.

A participant may be dropped from the study should a reported adverse reaction to the study supplement be considered severe.

G. Subject Safety and Data and Safety Monitoring Plan

Classification and Reporting of Adverse Events and Unanticipated Problems for This Study

An **Unanticipated Problem** is defined as: An incident, experience, or outcome that meets all of the following criteria:

1. The nature, severity, or frequency is unexpected for the subject population or research activities as described in the current IRB approved protocol, supporting documents, and the ICF(s).
2. It is related or possibly related to participation in the research.
3. It suggests the research may place the subject or others at a greater risk of harm than was previously recognized.

An **Adverse Event (AE)** is defined as: Any untoward or unfavorable medical occurrence in a human subject, including any abnormal physical exam or laboratory finding, symptom, or disease, temporally associated with a subject’s participation in the research.

AEs are classified by three categories:

1. Severity: *Non-serious* or *Serious*
2. Relation to Participation in Research: *Related, Possibly Related, or Unrelated*

The classification of the potential relationship to the intervention will be determined based on the following scheme:

Definite	Temporal pattern and known or expected adverse event response pattern, confirmed by stopping the intervention and reappearance of adverse event on re-challenge.
Probable	Temporal pattern and known or expected adverse event response pattern, confirmed by stopping the intervention and which could not be explained by the participant’s clinical state.
Possible	Temporal pattern and known or expected adverse event response pattern, confirmed by stopping the intervention and which could have been produced by a number of other factors.
Unknown	Relationship for which no evaluation can be made.
Not Related	Adverse event for which sufficient information exists to indicate that the cause is unrelated to the study intervention.

3. Expectation: *Expected* or *Unexpected* (determination is based on the known risks associated with the study supplement and procedures, natural progression of an underlying illness, and health characteristics of the study population)
4. Location: *Internal* (occurring at the HNRCA) or *External* (occurring at a site that is not under the jurisdiction of the Tufts MC/TUHS IRB)

Further, a **Serious Adverse Event (SAE)** is an **AE** that meets the following criteria:

1. Results in death, or
2. Is life-threatening, or
3. Results in hospitalization or prolongation of existing hospitalization, or
4. Results in a persistent or significant disability/incapacitation, or
5. Results in congenital anomaly/birth defect, or
6. May jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed above.

AEs are Unanticipated Problems only when they meet all three criteria in the definition of an unanticipated problem.

In the event of an Unanticipated Problem, or adverse event, staff will immediately notify the PI and study physician. Once notified, the PI, Dr. Mohsen Meydani, will take immediate action to eliminate or minimize risk to enrolled subjects. The study physician, Dr. Lisa Ceglia, will assess the severity of the unanticipated problem, and determine its relation to the study protocol. Each unanticipated problem will be reported, with all supporting documentation, according to the following IRB guidelines within 5 business days:

Information that does not fall under any of the categories does not require reporting to the IRB.

1. Harm experienced by a subject or other individual, which in the opinion of the investigator is **unexpected** and **related/possibly related** to the research procedures.
 - A. A harm is "**unexpected**" when its specificity and/or severity is **inconsistent** with risk information previously reviewed and approved by the IRB in terms of nature, severity, frequency, and characteristics of the study population.
 - B. A harm "**possibly related**" when there is a reasonable likelihood that the incident, experience, or outcome may have been caused by the procedures involved in the research.
- b. Information that indicates a new or increased risk, or a new safety issue. For example:
 - A. New information (e.g., an interim analysis, safety monitoring report, publication in the literature, sponsor report, or investigator finding) indicates an increase in the frequency or magnitude of a previously known risk, or

uncovers a new risk or greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

- B. An investigator brochure, package insert, or device labeling is revised to indicate an increase in the frequency or magnitude of a previously known risk, or describe a new risk
 - C. Withdrawal, restriction, or modification of a marketed approval of a drug, device, or biologic used in a research protocol
 - D. Complaint of a subject that indicates subjects or others might be at increased risk of harm or at risk of a new harm
 - E. Any changes or new information adversely affecting the conduct of the research (that indicates a new or increased risk, or a new safety issue)
- b. Non-compliance with the federal regulations governing human research or with the requirements or determinations of the IRB, or an allegation of such non-compliance
 - c. Audit, inspection, or inquiry by a federal agency and any resulting reports (e.g. FDA Form 483)
 - d. Any site monitoring or other audit report finding that directly and materially affects subject safety or their willingness to continue participation
 - e. Failure to follow the protocol that harmed a subject or placed subject at risk of harm (for example, missed study test or visit (or out of window) that could affect subject safety, enrollment of a subject who did not meet all eligibility criteria, failure to follow safety monitoring plan. These examples are intended as a guide and are not all-inclusive.)
 - f. Breach of confidentiality
 - g. Change to the protocol taken without prior IRB review to eliminate an apparent immediate hazard to a subject
 - h. Incarceration of a subject in a study not approved by the IRB to involve prisoners
 - i. Complaint of a subject that indicates that the rights, welfare, or safety of the subject have been adversely affected or a subject complaint that cannot be resolved by the research team
 - j. Premature suspension or termination of the protocol by the sponsor, investigator, or institution
 - k. State medical board actions against any member of the research team
 - l. [Unanticipated adverse device effect](#) (any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.)
 - m. Information that, per the sponsor, requires prompt reporting to the IRB

Data and Safety Monitoring Plan:

As this study is low-risk, it does not have a Data and Safety Monitoring Board (DSMB). The PI, along with the IRB, will ensure that volunteer safety and data integrity are maintained through our Data and Safety Monitoring Plan. This Data and Safety Monitoring Plan (DSMP) outlines the PI's, Dr. Mohsen Meydani, role as follows:

- Dr. Meydani will regularly monitor potential risks and procedures in order to protect participants from risk.
- Will discuss any adverse events with the study physician on a weekly basis.
- Will report any adverse events associated with participation in the study protocol and file all required annual paperwork with the IRB.
- Will evaluate the progress of the study including periodic assessments of data quality and timeliness, recruitment, enrollment and retention, protocol violations, unanticipated events, volunteer risk vs. benefit, and anything else that may have an impact on the study outcome.
- Maintain vigilance of developments outside of the study that could affect participant's safety.
- Perform, at minimum, two reviews of the data per year to ensure safety and efficacy of the study.
- Responsibility for all procedures performed.
- Reviews all data as it may relate to volunteer safety. This includes factors like participant retention and reason for drop-out.
- Reporting all serious adverse events regardless of their relation to the study protocol.
- Provide the IRB with summaries of all adverse events.
- At the discretion of Dr. Meydani, the Co-I, Dr. Roberts, or Dr. Saltzman, the study may be terminated at any time if the risks to study subjects are found to outweigh the potential benefits.

Additionally, no personal results will be communicated to study participants. However, participants will be informed of any necessary new findings developed during the course of this research study. Written results may be given directly, or mailed, to participants if they are still in the study, or mailed to them if they have completed their participation in the study.

H. Outcome

Our goal is to assess the safety and efficacy of EMIQ as it relates to body fat reduction. We predict that there will be no changes in health, and that there will be a significant difference between change in total body fat between the treatment and placebo groups.

I. Tissue Banking Considerations

Optional participation in tissue banking is offered during the informed consent process for participation in the main study. All pertinent information regarding what will happen to the stored samples, how long they will be stored; their potential future use for research, etc. will be outlined clearly. Participants will be informed as to how they can withdraw their sample from the tissue bank if they choose to do so. Participants will be asked to take the time to think about their decision, and will be encouraged to ask questions. All questions and concerns will be addressed to the participant's satisfaction to ensure the participant is giving informed consent.

J. References

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14. January 5, 2006 letter from Professor Shoji Fukushima to Professor Joseph F. Borzelleca regarding ADI (**See Supplementary Materials**)

15. Acute oral toxicity study of SANMELIN 11130 in rats, San-Ei Gen F.F.I., January 10, 1987 (**See Supplementary Materials**)

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Study Title: Body Fat Reducing Effect and Safety of Enzymatically Modified Isoquercitrin in Overweight and Obese Subjects
PI: Mohsen Meydani
Study Protocol
Version Date 3/2/2018

19. Answer for the peer review, Dr. Seiko Tamano, November 14, 2002
(Attached)

20. Bacterial reverse mutation study of enzymatically modified isoquercitrin (SANMELIN), San-Ei Gen F.F.I., Inc., December 19, 1986 **(See Supplementary Materials)**