MooDFOOD

PREVENTING DEPRESSION THROUGH FOOD

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	Adverse Event
CV	Curriculum Vitae
EFSA	European Food Safety Authority
DSMB	Data Safety Monitoring Board
EU	European Union
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
(S)AE	(Serious) Adverse Event
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Depression is one of the most prevalent, severe and disabling disorders in the EU and places a heavy burden on individuals and families. Additionally, a large proportion of the EU population is overweight, which according to previous research, increases the risk of depression. Recent research has suggested that there is a bi-directional link between healthy nutrition and psychological health. It is yet unclear whether nutritional behavior influences the development of depression in a direct manner or whether other mechanisms like social environment or obesity are also involved as well. The MooDFOOD consortium would like to gain a better understanding of the psychological, lifestyle and environmental pathways underlying the multi-faceted, bidirectional links of food intake, nutrient status, food-related behaviour and obesity with depression. The aim of the prevention study is to investigate whether two different nutritional strategies (a multi-nutrient supplement and food-related behavioural change) are feasible and effective in preventing depression in high-risk overweight EU citizens. This will be studied in four countries across Europe (the Netherlands, United Kingdom, Germany and Spain). It is hoped that improving food-related behavior and nutrient status may offer opportunities to prevent depression, especially in people prone to being overweight.

- ***Objective:*** To investigate whether two different nutritional strategies (a multi-nutrient supplement and food-related behavioural change) are feasible and effective in preventing depression over the course of one year in high-risk overweight EU citizens.

Study design: One-year long two-by-two factorial randomized placebo controlled prevention trial with two intervention conditions (a multi-nutrient supplement and a food-related behavioural change (FBC) intervention).

Study population: Volunteers aged 18-75 who are overweight and have mild depressive symptoms.

Intervention:

- Multi-nutrient supplement: comprising one capsule and one pill
  a) Omega 3 fatty acids: 1000 mg per capsule with a EPA/DHA ratio 3:1 (EPA > 700 mg per capsule, DHA > 100 mg per capsule)
  b) Multivitamin/minerals pill containing 100-200 mg calcium, 25-55 µg selenium, 400-600 µg B11-vitamin, 20 µg D-Vitamin.
- Placebo:
  a) Sunflower oil capsule with similar filling material and colour as the fatty acid capsule.
  b) Pill with inert substance/filling material with similar colour and shape as the multi nutrient pill as mentioned above.
- Food related behavioral change (FBC): The intervention will consist of 21 sessions (15 individual sessions lasting 30 minutes/each; 6 group-based hour-long sessions) with a trained therapist supervised by a gz-psychologist who will target the determines and idiosyncratic triggers of unhelpful (e.g., mood related snacking) and helpful food related behavior.

Main study parameters/endpoints:

- The 12 month cumulative incidence of Major Depressive Disorder (MDD), defined according to the standard psychiatric DSM-IV criteria using MINI (Mini International Neuropsychiatric Interview): baseline, 3, 6 and 12 months later[1].

- Depressive symptomatology according the Inventory of Depressive Symptomatology:[2;3] baseline, 3, 6, 12 months later.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

- Participants will be assessed at baseline, 3, 6, 9 and 12 months (4 site visits and one optional site visit at 9 months). This will involve an two hour long interview and anthropometric measurements. In addition, an online measurement will take place after 24 months.
- During the baseline assessment patients will be asked to fill in a questionnaire consisting of 12 topics
- There is an optional blood (not compulsory) draw which will be done at baseline, 6 and 12 months. It will involve 3 x 6ml & 2 x 8.5ml samples.
- Those allocated to the FBC will also need to attend 15 individual sessions (30 minutes) and 6 group sessions (60 minutes) of behavioural therapy spread over a period of 12 months with a therapist.
- All participants will be required to take a pill, either a placebo, or a multi-nutrient supplement every day for a year.

The contents of the multi-nutrient supplement will be similar to that available in over-the-counter supplements, hence well under maximum recommended daily dose, thereby presenting no health risks to the participants.

1.INTRODUCTION AND RATIONALE

Depression is one of the most prevalent diseases globally[4;5] and is currently the third leading contributor to the global disease burden, and estimated to rise to a first place ranking in 2030[6]. Lifetime prevalence of depression varies from 10%-25% in women and 5%-12% in men. About 6% of the population meets the criteria for major depressive disorder at any time[7]. The adverse health consequences are tremendous: meta-analyses indicate that depression is associated with premature death[8], cancer[9], diabetes[10] and cardiovascular disease[11]. Moreover, depression has a range of social and economic consequences, including stigma and discrimination, work absenteeism, and unemployment[12;13]. Hence, depression needs to be considered as an important risk factor for general public health and strategies to prevent the development of depression are urgently needed.

Although the exact etiology remains unclear, it is obvious from a range of studies that depression is a multi-factorial disorder: its risk is determined by a complex interplay of social, environmental and biological factors, including stressful experiences, genetics, nutrition, and hormonal actions on the brain. Recent studies have suggested a bi-directional link between healthy nutrition and psychological health. Observational studies show that the change from a more traditional diet to the consumption of more convenient, industrially prepared, processed foods (i.e. fried foods and refined grains) is associated with a higher risk of depression[14] whilst a healthy diet, comprising fruits, vegetables, fish and whole-grains is associated with less depression[5;15;16]. Studies trying to identify specific food groups associated with depression have found that a high fish consumption is associated with a reduced risk of depression[5;17;18]. On a nutrient level, epidemiological studies demonstrate that lower levels of vitamin D maybe associated with the onset of depression[19] and higher depression scores[20;21]. Additionally lower levels of the B-vitamin folic acid is also associated with depression[22].

Although depression is related to obesity, it is yet unclear whether nutritional behaviour influences development of depression in a direct manner. Observational studies have shown that depression can influence food choice via physiological effects that change appetite, or other behaviour that constrains or alters food availability[23] such as meal skipping, and more disordered eating[24;25]. Furthermore, stress-driven eating is associated with eating more energy-dense high-fat foods and obesity[26] and overall unhealthy diet choices, characterized by a greater intake of saturated fat, sodium, and sugar[27]. Disturbances in eating behaviours

and/or activation of stress-related hormonal pathways and pro-inflammatory cytokines which interfere with glucose metabolism may underlie these associations.

In summary, there are several indications showing that depression influences food-related behaviour and food intake. However, which food-related behaviours explain the link between food intake and depression remains largely unexplored.

A factor which can be included in a model linking food intake to depression is obesity, which meta-analysis has shown to be linked to depression[28]. Several biological mechanisms could explain this relationship such as the fact that obesity can perhaps be seen as an inflammatory state which activates inflammatory pathways[29] or obesity may be involved in the dysregulation of the hypothalalmus-pituitary-adrenal axis[30]. Furthermore, obesity involves increased risks of diabetes and increased insulin resistance[31] which could induce brain alterations and increase the risk of depression[32]. Finally, very recent studies suggest that obesity predisposing genes may increase the risk of depression[33]. In addition to biological mechanisms, psychological pathways should be mentioned. Being overweight, and the perception of being overweight, increases psychological distress[34]. In both the United States and Europe thinness is considered a beauty ideal, and partly due to social acceptance and sociocultural factors, obesity may increase body dissatisfaction and decreased self-esteem which are risk factors for depression. Based on this previous research, psychological, lifestyle and environmental pathways as well as genetic pathways may underlying the link between obesity and depression and need to be further determined to allow the development of effective strategies for the prevention of both obesity and depression.

As of yet, few experimental studies have been conducted to investigate whether specific food characteristics and food-related behaviours can prevent depression. Meta-analyses showed a positive effect of n-3 long-chain unsaturated fatty acid supplementation, and particularly eicosapentaenoic acid in individuals with a diagnosis of depression[35] and another meta-analysis of RCTs confirms that supplementation of eicosapentaenoic acid was effective against primary depression[36]. Moreover, recent trials confirm that high doses of n-3 fatty acids improve depression scores[37;38], although some showed no effect[39]. While these results are promising, as of yet no randomized, long-term controlled trials investigated the effect of n-3 fatty acid supplements on the prevention of depression.

Several intervention studies showed that vitamin D supplementation enhanced mood scores[40] and improved depressive symptoms in obese persons[41]. However, other RCTs showed no beneficial effect of supplementation[42-44]. Although the evidence is mixed, there are some studies that provide mechanistic support for the beneficial role of vitamin D in

depression.[45]. Vitamin D receptors are present in several brain regions which are associated with depressive disorders[46] , and cells in many of these regions are capable to metabolize the inactive form of vitamin D to its active form[46]. Whether vitamin D is a likely candidate to be effective in the prevention of depression remains to be determined. B vitamin supplementation, including folic acid, may reduce the long-term risk of onset of depression via reduction of vascular and other metabolic risk factors for late-life depression[47] . Supplementation with folic acid, vitamin B12 and B6 was shown to reduce major depression in some[48] but not all studies[49;50]. Based on the scientific literature thus far, it remains unclear whether supplementation with folic acid and other B vitamins may prevent the development of depression.

Based on these previous studies a conceptual model (Figure 1) has been created to unravel the different pathways underlying the bidirectional link of food intake, nutrient status and food-related behaviour with food intake and food-related behaviour include food choice, food intake (including alcohol consumption), food patterns, food-related practices (meal preparation, food provisioning), and psychological eating behaviour.

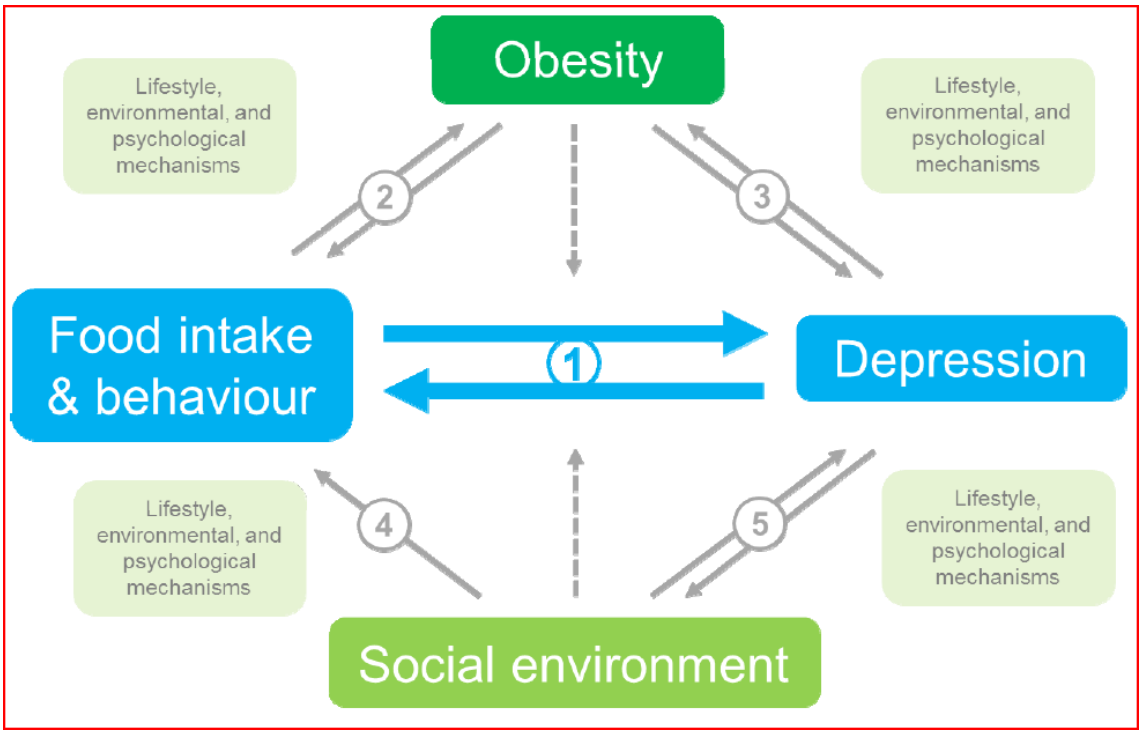


Figure 1: Conceptual model of relationships between food intake and behaviour and depression.

In order to understand the role nutritional status and food related behavior play in the development of depression long-term intervention studies are needed. None of the previous

trials have investigated both the effect of a nutrient supplementation and healthy eating behaviours on the development of depression.

This trial will investigate whether two different nutritional strategies (a multi-nutrient supplement and food-related behavioural change) are feasible and effective in preventing depression. This will be done in a population who is at risk of developing a major depressive disorder (people who already have depressive symptoms). Furthermore, because depression may possibly be linked with obesity, and weight maybe an important contributing factor to the role of food intake and food related behaviour on the development of depression, this study will enrol participants who have a body mass index of more than 25.

2. OBJECTIVES

Primary Objective: To investigate whether two different nutritional strategies (a multi-nutrient supplement and food-related behavioural change) are feasible and effective in preventing depression over the course of one year in high-risk overweight EU citizens, specifically citizens in the United Kingdom, The Netherlands, Germany and Spain.

Secondary Objective:

To investigate the sustainability of two nutritional strategies (a multi-nutrient supplement and food-related behavioural change) in overweight EU citizens with depressive symptoms.

To investigate the impact of two nutritional strategies (a multi-nutrient supplement and food-related behavioural change) on secondary outcomes (food-related behaviour, food intake and costs, body weight, physical activity, sedentary behaviour, depressive symptom scores, body image and food perception, and quality of life) in overweight EU citizens with depressive symptoms.

To examine mediators and moderators of the (potential) effect of two nutritional strategies in preventing depression in overweight EU citizens with depressive symptoms.

To investigate the effects of the two nutritional strategies (multi-nutrient supplements and food-related behavioural change) on long-term outcomes of depression, food intake and food-related behavior and other secondary outcomes at 24 months in high-risk overweight EU citizens.

3. STUDY DESIGN

One-year long two-by-two factorial randomized, placebo-controlled, prevention trial with two intervention conditions.

1) A multi-nutrient supplement (in the form of a pill and a capsule)
2) Food-related behavioural change (FBC) intervention

Table 1: Distribution of participants over the 4 potential intervention groups

	Food-related behavioural change therapy	No Behavioural change therapy
Multi-nutrient supplement	250 (±63 in NL)	250 (±63 in NL)
Placebo pill	250 (±63 in NL)	250 (±63 in NL)

Placebo pills will be given to the control group and both participants and researcher will be blinded to which participants receive the multi-nutrient pill and which the placebo.

This trial is part of the larger EU project MooDFOOD (www.moodfood-eu.vu). The intervention part of MooDFOOD project will be carried out in four different European countries: the United Kingdom, the Netherlands, Germany and Spain. A total of 250 participant will be enrolled in each country. The data will be pooled giving a total of 1000 participants. The study protocol is similar in all sites, differing only in the language of the questionnaires and the extra legal requirements that are specific to the individual countries.

The Dutch arm of the study will be conducted at VU University Medical Center in Amsterdam. It will run totally for 36 months, including an inclusion period of one year in which patients are recruited and randomized. Once enrolled patients will be followed for a period of two years (after 3, 6, 12, and 24 months).

4.STUDY POPULATION

4.1 Population

Participants will be enrolled from the general population, from general practitioners and from existing study cohorts from around Amsterdam. The recruitment of participants will take place between July 2015 and June 2016.

4.2 Inclusion criteria

- Age 18- 75 years
- BMI (Body mass index) 25-40
- PHQ-9 (a measure of mental health) ≥5 (mild depression)

4.3 Exclusion criteria

- Current (in past 6 months) clinical Major Depressive Disorder Episode (according to psychiatric DSM-IV criteria as determined with the MINI).
- Current (in past 6 months) use of antidepressant drugs or psychological interventions.
- History of psychosis, schizophrenia, bipolar disorder, or eating disorders. This will all be measured by a telephone screening interview.
- Anxiety or alcohol or substance/drug addiction in previous 6 months
- History of bariatric surgery, current severe life-threatening disease, or severe cognitive impairment limiting the conduct of the study, as assessed through research staff- evaluation of feasibility of conducting the screening instruments in an adequate manner.
- Currently adhering to supervised behavioral interventions that intervene with MooDFOOD interventions.
- Current use of dietary supplements that are competing with the MoodFood intervention. (Persons must be willing to stop what they were using before the start of this study, or will be excluded if dietary supplements were prescribed by a GP)
- Pregnancy or breastfeeding

4.4 Sample size calculation

The trial is powered to detect a difference of 25% in the cumulative incidence rates of DSM-IV depressive disorder between the conditions across 1 year. The 12-month cumulative incidence is expected to be 30% in the control condition (placebo, no FBC intervention) and 15% in the intervention group on the basis of longitudinal studies[51;52]. Because we were also interested to test for additive interaction of the two intervention strategies, we calculated that 180 participants per group would be needed, assuming a 2-sided test at α=.05 and a power of $(1 - \beta)$=0.90. Assuming a drop-out of 20%, a total of 250 subjects per intervention arm is

sufficient. Power will be much larger for the IDS-30 continuous outcome measures. A previous depression prevention trial has indicated that smaller number of subjects could already result in significant differences[53]. If the treatments turn out to be synergistic rather than additive the pure treatment groups will be compared and then the power to detect a difference on the main outcome will be 80%.

5. TREATMENT OF SUBJECTS

5.1 Multi-nutrient pills

Participants will be treated with either a multi-nutrient supplement (a pill and a capsule) or a placebo pill and capsule the contents of which are detailed below. The multi-nutrient supplements or placebo pills needs to be taken every day during one year. All subjects will be asked to refrain from using other vitamin supplements that comprise omega-3 fatty acids, Vitamin D, Calcium, Selenium and B11 (folic acid) for the study duration.

Intervention and placebo pills

1. **Multi-nutrient supplement:**
   The multi-nutrient supplement comprises two pills which need to be taken every day for the duration of one year.

   a. Omega 3 fatty acids: 1000 mg per capsule with a EPA/DHA ratio 3:1
   Content:
   - EPA content exceeding 700 mg per capsule
   - DHA content exceeding 100 mg per capsule

   Pharmaceutical form
   - Soft gelatine capsule of non-porcine origin
   - Enteric-coated capsule to prevent backflow

   b. Multivitamin/minerals
   Content:
   - Calcium: 100-200 mg
   - Selenium: 25-55 µg
   - B11-vitamin: 400-600 µg
   - D-Vitamin: 20 µg

   Pharmaceutical form
   - Multinutrient Pill

2. **Placebo:**
   a. Sunflower oil capsule with similar filling material and colour as the fatty acid capsule as mentioned above
   b. Pill with inert substance/filling material with similar colour and shape as the multi nutrient pill as mentioned above.

Both types of pill (multi-nutrient and placebo) will be distributed at baseline, 3, 6, 9 months whilst attending the initial and follow-up interviews. Participants will be telephoned 2 weeks after the baseline assessment to check that they are not having any problems with taking the pills. Participants will return all pill bottles with any unused pills at the follow-up

measurements where a researcher will weigh the bottles to see how many pills were returned. Unused pills will be destroyed. Participants will also be asked to complete an adherence questionnaire every 3 months.

5.2 Behavioural therapy

The intervention will consist of 21 sessions (15 individual sessions, 30 minutes/each; 6 group-based sessions, 1 hour/each). The intervention will be supervised by a gz-psychologist and will include detailed analysis of each individual's behavior to determine idiosyncratic triggers and functions of unhelpful (e.g., mood-related snacking) and helpful food-related behavior, thereby, to reinforce helpful behaviors and to implement effective alternatives to unhelpful behaviors, building on behavioral approaches proven effective in depression. A Behavioural Activation therapist who will receive a specific training in Exeter on food and food behavior will deliver the intervention. A dietician will be available for consultation.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational products

The investigational product is a multi-nutritional supplement which takes the form of a vitamin pill and a capsule. A placebo, inert version, shall be used as a reference intervention.

The exact compositions of the Omega-3 fatty acids, multivitamin/minerals, and both placebos, can be found in the PDF file C1.2 with detailed descriptions of the food supplements requirements (p.3 – p.12), as well as in the text box below.

Composition of Multi-nutrient Supplements

Omega-3 PUFA fish oils

Contains fish oil (minimum 65% Eicosapentaenois acid EPA, minimum 10% docosahexaenoic acid DHA), gelatine, water, glycerine, pectin, sorbitol, mixed of natural tocopherols and ascorbyul palmitate

Omega-3 placebo

Contains sunflower oil 78 (40%), gelatine and glycerine.

Multivitamins/minerals

Contains microcrystalline cellulose, calcium, carbonate, corn starch, polyvinylpyrrolidone, crosslinked carboxymethylcellulose sodium, coating agent, magnerium stearate, magnesium silicate, folic acid, sodium selenite and vitamin D3 .

Multivitamins/minerals placebo

Contains microcrystalline cellulose, calcium, carbonate, corn starch, polyvinylpyrrolidone, crosslinked carboxymethylcellulose sodium, coating agent, magnerium stearate and magnesium silicate

Flucctuation: Acceptable devition 90%-125%

The Spanish manufacturer Ferrer Internacional S.A. will be responsible for manufacturing, distribution and transformation of all the supplements. The manufacturing authorizations as well as a detailed description of the supplements' toxicological information with regard to allergies and shelf life can be found in document C1.2 on p. 13-18 and p. 20 – 23.

6.2 Summary of findings from clinical and non-clinical trials

MooDFOOD will determine whether multi-nutrient supplements can prevent the development of depression. The supplement will contain nutrients for which deficiency has been linked to depression risk in prior (pre)clinical studies. Lack of e.g. vitamin D, B-vitamins, antioxidants and omega-3 fatty acids have shown to contribute to reduced synthesis of synaptic membrane phosphatides essential for neuronal growth and function and synaptic and brain plasticity thereby impacting on depressive symptomatology. In support of this, epidemiological studies suggest that a diet rich in omega-3 fatty acids, vitamin D, B-vitamins and antioxidants decreases the risk of depression, while other studies suggest that patients with depression have lower plasma levels of these nutrients when compared to non-depressed subjects. These observations suggest that a multi-nutrient supplement containing various nutrients may be an efficient nutritional strategy to prevent depression. The final selection of the content of the supplements was based on the literature as part of Work Package 1 of the MooDFOOD project. With respect to the dose, we do not want provide 'super dosages' but adhere to dietary guidelines and target supplementing deficiencies only. Below the summary of research findings of the included multinutrients are shown.

Omega-3 PUFA fish oils

High fish consumption could be one explanation for the reduced risk of depression associated with whole food dietary patterns[5;17;18].Omega 3 long chain effect of fish consumption on depression[54;55] by modulation of serotonergic neurotransmission [56]. Furthermore, depressive patients have often low circulating levels of n-3 fatty acids because of abnormalities in their metabolism[55].

Meta-analyses showed a positive effect of n-3 supplementation, and particularly eicosapentaenoic acid (EPA) in individuals with a diagnosis of depression[35;57;58]. Another meta-analysis of RCTs confirms that supplementation of EPA was effective against primary depression[55]. Moreover, recent trials confirm that high doses of n-3 fatty acids improve depression scores[37;38], although some showed no effect[39] .

Vitamin D

Humans derive 80 to 90% of their vitamin D via endogenous synthesis in the skin and consequently lack of outdoor life has severe impact on vitamin D levels. As exposure to sunlight confers an increased risk of skin cancer[59;60], different ways to improve the vitamin D status are needed since in most western societies the vitamin D intake is not sufficient[61]. Based on a limited number of epidemiological studies, lower levels of vitamin D may be associated with the onset of depression[19;62] and with higher depression scores[20;21;63]. Several

intervention studies showed that vitamin D supplementation enhanced mood scores[40] and improved depressive symptoms in obese persons[41]. However, other RCTs showed no beneficial effect of supplementation[42-44;64]. The positive results are however supported by mechanistic studies[45]. Vitamin D receptors are present in several brain regions which are associated with depressive disorders[65], and cells in many of these regions are capable to metabolize the inactive form of vitamin D to its active form[65;66].

B-vitamins

The B vitamin folic acid or otherwise named folate – which is mainly found in green vegetables, fruit, beans or whole-wheat products – is also associated with depression. Persons with lower intakes of folate were more likely to be diagnosed with depression and to experience recurrent depressive episodes[22;70;71]. In addition B vitamin supplementation may reduce the long-term risk of onset of depression via reduction of vascular and other metabolic risk factors for late-life depression[47]. Supplementation with folic acid, vitamin B12 and B6 was shown to reduce major depression in some[48] but not all studies[49;50]. Based on the scientific literature thus far, it remains unclear whether supplementation with folic acid and other B vitamins may prevent the development of depression.
Long-term depression prevention trials focusing on food-related behaviour and the diet as a whole are currently lacking, and therefore MooDFOOD is going to carry out such a trial.

Calcium

Calcium and vitamin D are undoubtedly essential nutrients for the human body. A study by Bowden et al[73] investigated extracellular and intracellular calcium in patients with unipolar, bipolar or manic patients. They found that plasma calcium levels were decreased in unipolar and manic patients compared to controls, unipolar patients had lower plasma calcium concentrations compared to bipolar patients, and red blood cell calcium ATPase (an enzyme that is responsible for the removal of calcium from the cytosol) was lower in patients with unipolar depression.

Selenium

Depression is significantly associated with selenium blood levels[75;76] and low levels of dietary selenium are associated with an increased risk for a major depressive disorder[77]. A number of studies even suggest the protective factor that selenium supplementation provides by improving depressive symptoms among patients with low selenium levels[78], although not all studies show evidence that selenium supplementation benefited mood[79].

6.3 Summary of known and potential risks and benefits

Subjects randomized to the placebo condition will receive a placebo pill. The multi-nutrient supplement or placebo pill needs to be taken every day during one year. MooDFOOD will be

the first multi-country study to provide feasible and effective nutritional strategies to prevent depression in EU citizen. By providing a multinutrient that 1) targets supplementing deficiencies only 2) is consumed in dosages which are widely available over the counter of local pharmacies and/or drugstores 3) contains dosages that adhere to dietary guidelines in all countries, we provide our participants the opportunity to extend their use of a multivitamin even after their participation in our trial is over.

Based on the current scientific knowledge in the field of depression, the supplement will include vitamin D, omega-3 fatty acids, minerals and B-vitamins. As deficiencies of these nutrients are related to osteoporosis, cardiovascular diseases, and congenital spinal deformities, the benefit of these multi-nutrient supplements for society are reaching further than prevention of depression alone and will benefit overall health and well-being of EU citizens.

Omega-3 PUFA fish oils

Omega-3 fatty acids and EPA can improve depression scores. The intended dose (EPA+DHA =1.0g per day) of the PUFA fish oils are at a level that is freely available over the counter. The level of EPA and DHA in the supplement is well below the amount of 3g of omega-3 fatty acids per day that the FDA[81] considers safe and even more below the 5 g/d of a combination of EPA and DHA that European Food Safety Authority (EFSA) considers a safe level for adults[82].

Vitamin D

Vitamin D is deficient in many individuals and extra supplementation will correct this deficiency. Vitamin D is maybe associated with an improvement in depression scores. The dose of vitamin D administered in the MooDFOOD trial is still at a level that is freely available over the counter and is considerably lower than the dose of vitamin D supplementation used in studies that showed vitamin D intoxication and related hypercalcemia. DeLuca[67] concluded that, overall, the toxicity of hypercalcemia becomes evident at vitamin D intakes above 25,000 IU/day and Hathcock et al.,[68] following an analysis of more than 20 publications, concluded that there was no association between harm and intakes of 10,000 IU/day. The level of vitamin D in the MooDFOOD supplement (20 µg or 800IU) is also well below the 4000IU per day that the IOM (IOM 2011) considers as the safe upper limit and even more below the 100 µg per day that EFSA considers a safe level for adults[69].

B-vitamins

In the MooDFOOD trial the B vitamin folate will be administered at the dose of 400-600 µg per day, which dose is close to the recommended intake for adults in Europe ranging between 200 and 400 microgram of folate per day. The dose of folate administered in the

MooDFOOD trial is still at a level that is freely available over the counter and is considerably lower than the tolerable upper limit of 1000 µg per day by EFSA.[72]

Calcium

The level of calcium in the MooDFOOD supplement is well below the safe upper limit of 2500mg per day by the IOM[74] for adults 19-50 years of age and the 2000 mg of calcium per day for persons 51 to 70 years of age and for persons more than 70 years of age.

Selenium

Selenium supplementation can be harmful to an organism due to its tight therapeutic range, but in the MooDFOOD trial selenium will be administered at the dose of 25-55 µg per day. With this dose the selenium levels are still at a level that is freely available over the counter and even below the adequate daily intake of 70 µg per day for adults and the 300 µg that EFSA considers a tolerable upper intake level for adults.[80]

6.4 Description and justification of route of administration and dosage

The selected nutrients may thus be an efficient nutritional strategy to prevent depression. The supplements will be provided in capsules (omega-3 fatty acids) and pills (all other nutrients) that will be taken daily for a year. All dosages are well below the EFSA levels of tolerability, and adhere to dietary guidelines of the four countries where the trial will take place. Moreover the supplements, like over the counter supplements, will make allowances for the fact that an individual may actually already have a high level of the nutrient in question. Hence, participants with naturally occurring high levels (due to diet) of a nutrient will not exceed the recommended maximum daily allowance even when the multi-nutrient supplements are added to their daily intake.

Omega 3 -PUFA fish oils

In the MooDFOOD trial the omega 3 PUFA fish oils will be administered in capsules at the dose of >700mg EPA and >100mg DHA per day, reflecting recent meta-analyses indicating that doses in the range 0.6-4.4g EPA plus 0.2-2.2g DHA , with EPA dose greater than DHA, were efficacious relative to placebo in reducing depressive symptoms in patients with depression[36;57].

Multinutrient pill

Vitamin D

In the MooDFOOD trial the Vitamin D will be administered at the dose of 20µg (800IU) per day.

B-vitamins

In the MooDFOOD trial the B vitamin folate will be administered at the dose of 400-600µg per day, which dose is close to the recommended intake for adults in Europe ranging between 200 and 400 microgram of folate per day.

Calcium

Evidence supports the use of calcium, or calcium in combination with vitamin D supplementation, in the preventive treatment of osteoporosis in people aged 50 years or older. Because vitamin D is often co-prescribed with calcium, and MoodFood participants maybe discontinuing their calcium supplements, calcium will also be included in the tablets. In the MooDFOOD trial calcium will be administered at the dose of 100-200mg per day.

Selenium

In the MooDFOOD trial selenium will be administered at the dose of 25-55 µg per day.

Preparation and labelling of Investigational Medicinal Product

A detailed description of the manufacturing process of both the Omega 3 supplements, multivitamins/minerals and placebos is provided in document C1.2 on p. 24 – 27. The steps, components, description of the operations performed, equipment needed and controlled parameters are described in flowcharts. Ferrer International will submit the certificates of analysis and release batch analysis reports with the delivery of the products.

On p. 4-12 of the document C1.2, the official labels can be found, providing information regarding the ingredients, usage mandatory obligations in accordance to the product legal status. On p. 31 and 32,and below are examples of the labels that will be used for patient information are shown, with short instructions on storing, usage, expiry date and the MooDFOOD logo.

6.5 Drug accountability

All multi-vitamin pills and capsules will be shipped to Amsterdam where they will be labelled and given a unique code by an independent person (i.e. somebody who is not directly involved with the recruitment and assessment of patients. A 13 week supply of multi-nutrient supplements will be given to participants at the baseline interview, and at months 3, 6 and 9. When participants return for their follow-up measurement they will be asked to bring back the Multi-nutrient bottles along with any unused pills and capsules. These will then be weighed (to calculate how many pills/capsules were not consumed) and destroyed.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

8. METHODS

8.1 Study parameters

An overview of the measurements and their time of assessment can be found in table 2.

8.1.1 Main study parameter/endpoint

- The 12-month cumulative incidence of Major Depressive Disorder (MDD), defined according to the standard psychiatric DSM-5 criteria using The MINI V5.0[1] instrument. This will be measured at baseline, 3, 6 and 12 months later.
- Depressive symptomatology according the Inventory of Depressive Symptomatology (IDS)[2;3] : baseline, 3, 6, 12 months later

8.1.2 Secondary study parameters/endpoints

- Food intake (FFQ-GA2LEN)[83]
- Food behaviour and sustainability: Food Behavior Questionnaire (Developed by MooDFOOD researchers)
- Food and eating behaviour: The Three-factor Eating Questionnaire (TFEQ-18; Karlsson et al., 2000)
- Physical activity and sedentary behaviour with validated questionnaires (Short Questionnaire to Assess Health - Enhancing Physical Activity (SQUASH, Wendel-Vos 2003) and sedentary behaviour (SBQ[84])
- Body weight perception: The Stunkard figure rating scale (Stunkard et al.,1983)
- Generalized Anxiety Disorder Assessment (GAD-7; Spitzer et al, 2006) for anxiety symptoms
- Quality of life (EuroQol instrument, EQ-5D-5L, EuroQol group, 1990)
- Automaticity of good and bad health behaviours and compliance to Mediterranean diet (Self-reported behavioural automaticity index (SRBAI)[85])
- Behavioural functionality: activation, avoidance/rumination, work /school impairment and social impairment (The behavioural activation for depression scale (BADS)[86])
- Evaluation of supplements
- Evaluation of lifestyle coaching
- LIDAS (depression)[87] after 24 months

8.1.3 Other study parameters

Other study parameters will be included as potentially confounding or modifying variables. These include:

- Body mass index, waist and hip circumference
- Blood pressure
- Marital status
- Education level
- Work status
- Income
- Smoking status
- Alcohol use
- History of chronic illness
- Depression history of family
- Medication use
- DNA
- Biomarkers of nutritional status
- Menstrual cycle (women only)

Furthermore, we measured the depression outcomes and all secondary outcomes (except the evaluation questions) also after 24 months to be able to study the long-term effects and course.

8.2 Randomisation, blinding and treatment allocation

For all study centres a centralised online random generator will be used to block randomise the participants stratified by participants with a history of depression to ensure that the distribution of these participants are evenly distributed to each group. Participants will also be stratified according to study centre. Both participants and researchers will be blinded to the receipt of the placebo or multi-nutrient pill.

8.3 Study procedures

Participants will be predominantly enrolled by mass mailing letters. This will be done primarily by approaching a few local councils in the area surrounding VU University Medical Center, Amsterdam, and asking them for a mailing list of all residents between the ages 18-65. Depending on the recruitment rate, more councils will be approached as the study proceeds. The letters sent to residents will be accompanied by an information brochure and will invite potential participants to go to the MoodFOOD website where they can check whether they fall within the main eligibility criteria (BMI >25 and PHQ-9 ≥5). Eligible participants will automatically be invited to leave a telephone number and email address on the website which will be forwarded to the study centre. Potential participants will also have the option of

completing a paper version of the PHQ-9 which they can return to the study centre via a pre-paid envelope. Secondary recruitment strategies include mailing via GP practices, unaddressed local postal mailings, posters and flyers that will be used in general practitioner settings, community centers, paramedical centers, sport centra, shopping centers, university,(social) media and the internet, all of which will direct potential participants to the MooDFOOD website where they can read about the study, check their eligibility and contact the study centre. Participants who are eligible or who pass the on-line screening will be contacted by telephone for a further screening process in which all the eligibility criteria will be checked along with their understanding of the study and what it will involve. Following a successful telephone screening an appointment will be made for a baseline assessment. A flow diagram depicting the enrolment and screening of participants can be seen in figure 2. There is a period of at least one week between the telephone screening and the baseline interview, in which participants can decide whether they really want to participate.
During the baseline assessment the participant will attend the study centre at VUmc Psychiatry (location A.J. Ernststraat). First an informed consent will be signed. The baseline assessment will also involve an interview, in which depression history, socio-demographic, lifestyle and medical history data will be collected, a physical assessment (length, body weight, waist and hip circumference and blood pressure measurements), and a blood draw (optional) to measure cholesterol, carotenoids and fatty acid concentrations. A biobank will be established in the Netherlands from which compliance to the therapy, biological changes due to the nutritional supplements can be determined. Furthermore genetic material (Deoxyribonucleic acid (DNA)), and epigenetics will be analysed. The participants will also be invited to complete 12 self-reported questionnaires on a computer at the study centre. They may also complete this task at home in their own time.

During the completion of the questionnaires the participants will be randomised to one of the four treatment arms. The participant will then be given their pill package (a 4 month supply) with instructions. Participants allocated to the food-related behavioural change therapy will be telephoned within 2 weeks to make an appointment with the therapist.

At week 2 a blinded researcher will telephone the participant to see if there are any problems taking the pills or capsules. Follow-up measurements will take place at month 3, 6 and 12. This assessment includes comparable measurements as the baseline assessment (table 2). Participants will be reimbursed for travel costs for visits associated with baseline and follow-up assessments. In addition, after 24 months, an online follow-up will take place.

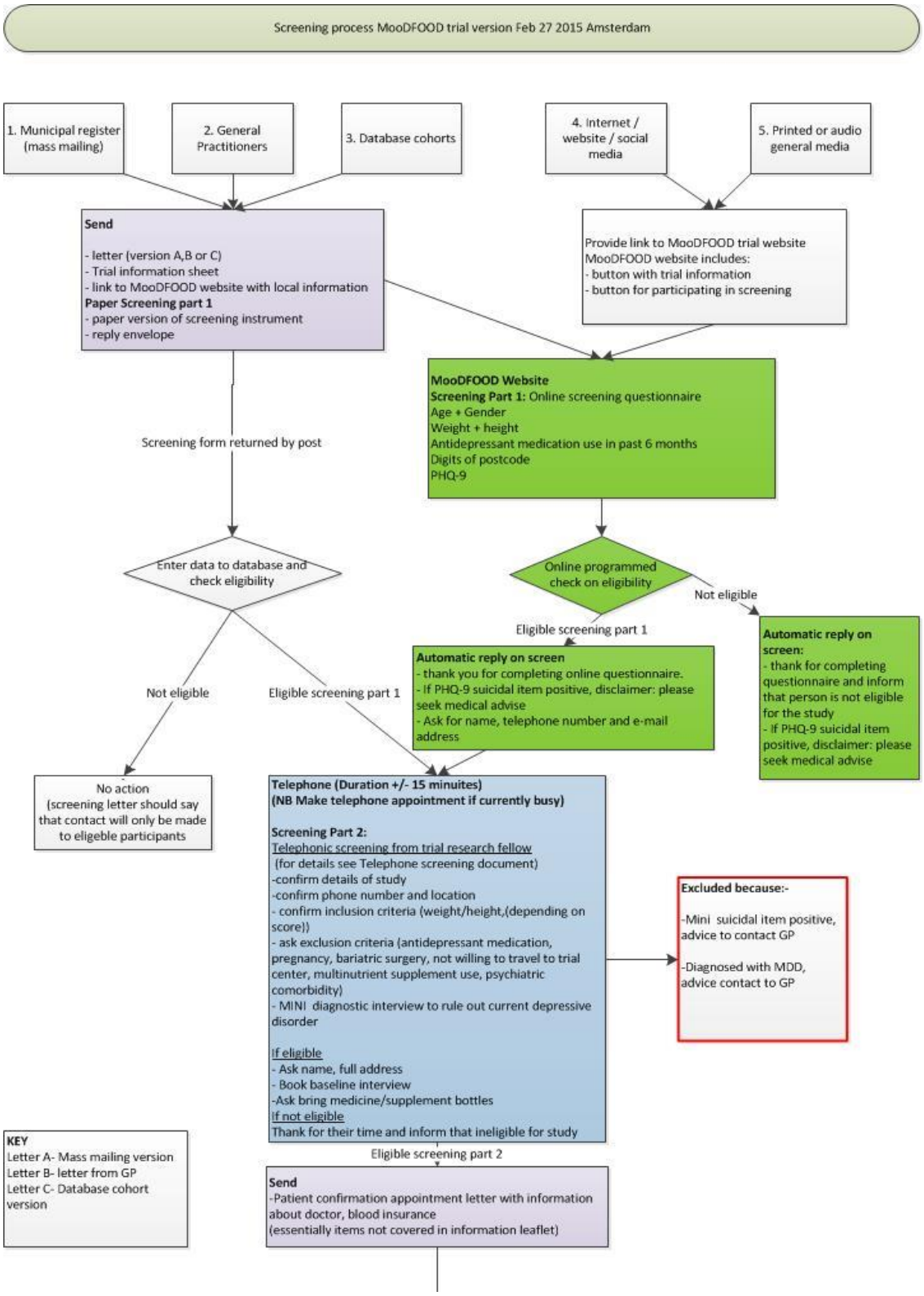
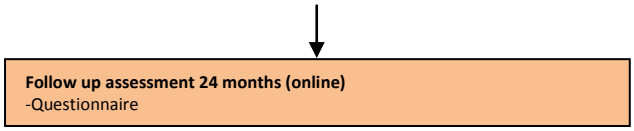


Figure 2: *Flow diagram depicting the recruitment and screening process*

Figure 3: Flow diagram depicting the measurement process



INSTRUMENT	MAIN VARIABLES	TIMELINE	
Screening		**Online/ paper screening**	**Telephone Screening**
Other screening questions online	-Age, -gender, -self-reported weight, -self-reported height, -antidepressant medication use past 6 months, -post-code digits	X[q]	
PHQ-9 (Patient Health Questionnaire)	Screening of mental health disorders: mood	X[q]	
Other screening questions telephone	-Self-reported weight, -self-reported height, -antidepressant use past 6 months, - psychological therapy in past 6 months -pregnancy current or planned in next year, -bariatric surgery in next year, -is prepared to travel to study centre for visits -is prepared to stop current use of vitamin and/or mineral supplements -self-reported severe / life-threatening disease -self-reported diagnosis of bipolar disorder (ever), schizophrenia or psychosis (ever), eating disorder (ever) - self-reported diagnosis and treatment in past 6 months for: anxiety disorder, alcohol substance/drug addiction (ever and current) - cognitive impairment (evaluated by interviewer) - date of birth - address		x[i]
MINI (V5)-Present MDD	Assessment of MDD according to the definitions/ criteria of DSM-IV		x[i]

Interview		Baseline	3 months	6 months	9 months	12 months
Informed Consent		x				
MINI (V5)-Present MDD	Assessment of MDD according to the definitions/ criteria of DSM-IV		x[i]	x[i]		x[i]
MINI (V5)-Lifetime MDD	Historical assessment of MDD according to the definitions/ criteria of DSM-IV	x[i]				
Socio-demographics	Sex, Date of Birth, civil status, housing, education, job, income	x[i]				
Smoking	Smoking status	x[i]		x[i]		x[i]
AUDIT	Alcohol disorders	x[i]				x
Chronic Illness	History of Chronic illnesses	x[i]				
Chronic Illness follow up	Has there been a change in health in the previous 3/6 months		x[i]	x[i]		x[i]
Depression history of family		x[i]				
Medication/supplement use	Medications used in last month (INCLUDING multi-vitamin/nutritional supplement use)	x[i]				
Medication/supplement use follow-up	Medication used in last six months (INCLUDING multi-vitamin/nutritional supplement use)			x[i]		x[i]
Menstrual cycle						x[i]
Positive/negative effects of supplements				x[i]		x[i]
Other interventions	Assessment of other diet-related or psychological programs/interventions during the MooDFOOD trial					x[i]
Physical Measurements		**Baseline**	**3 months**	**6 months**	**9 months**	**12 months**

Blood pressure		x	x	x	x*	x
Anthropometrics	Weight, height, waist circumference, hip circumference	x	x	x	x*	x

Questionnaires (To be completed digitally (where possible) partially at study centre and finished at home)								
INSTRUMENT	**No items**	**MAIN VARIABLES**	**Baseline**	**3 months**	**6 months**	**9 months**	**12 months**	**24 months**
PHQ-9 (Patient Health Questionnaire)	9	Screening of mental health disorders: mood	X[q]	X[q]	X[q]		X[q]	X[q]
IDS-SR30 (Inventory of Depressive Symptoms - Self-Rated)	30	Depressive symptoms	X[q]	X[q]	X[q]		X[q]	X[q]
GAD-7 (Generalized Anxiety Disorder)	7	Anxiety symptoms	X[q]	X[q]	X[q]		X[q]	X[q]
Euro-Quol-5D-5L (EuroQol)	5	Health-related quality of life	X[q]	X[q]	X[q]		X[q]	X[q]
TFEQ-18(The Three-factor Eating Questionnaire)	21	Eating behaviour: cognitive restraint (CR), uncontrolled eating (UE) and emotional eating (EE)	X[q]	X[q]	X[q]		X[q]	X[q]
Food behaviour and Sustainability (instrument WP1/2)	47	Meal pattern, Practices, cooking and shopping, Mindful eating, Sustainability	X[q]	X[q]	X[q]		X[q]	X[q]
FFQ-GA2LEN	264	Food frequency questionnaire measuring food intake	X[q]		X[q]		X[q]	X[q]
The Stunkard figure rating scale	2	Body weight perception (Body image and perceived body size)	X[q]	X[q]	X[q]		X[q]	X[q]
SQUASH (Short Questionnaire to assess health-enhancing physical activity)	20	Physical activity	X[q]	X[q]	X[q]		X[q]	X[q]

SBQ-Sedentary behaviour	18	Sedentary behaviour	X[q]	X[q]	X[q]		X[q]	X[q]
SRBAI (Self-Report Behavioural Automaticity Index) x 3 (change open A & B and diet fixed)	36	Automaticity of good and bad health habits and Mediterranean diet	X[q]	X[q]	X[q]		X[q]	X[q]
BADS (The Behavioural Activation for Depression Scale)	25	Behaviour functionally: Activation, avoidance/Rumination, Work/School Impairment, and Social Impairment	X[q]	X[q]	X[q]		X[q]	X[q]
Adherence questionnaire	3	Moodfood developed questionnaire about nutritional supplement adherence		X[q]	X[q]	X[q]	X[q]	
Evaluation of supplements	7	Evaluation of supplements for 12 month period					X[q]	
Evaluation of lifestyle coaching	25	Evaluation of lifestyle coaching for 12 month period					X[q]	
LIDAS (modified for MooDFOOD)ref	26	Evaluation of depressive episodes since past interview (12 months)						X[q]
Weight (change) items from LASA study	8	Weight and weight change in past year						X[q]
Supplement use	9	Supplement use in past year						X[q]

Blood	Purpose	Baseline	3 months	6 months	9 months	12 months
Serum (2 x 8.5 ml)	Biobank	x		X		X
Serum (: 6ml)	Biobank: trace elements	x		X		X
EDTA (2 ml)	Biobank: hematology	X		X		x
EDTA (6 ml)	Biobank: plasma	X		x		x
EDTA (6 ml)**	DNA isolation	X		x		x

[q]= Measure with self-reported questionnaire
[i]=Asked during interview

*For those who want to visit the research center to exchange supplements, we do blood pressure and antropometric measurements to improve study participation. For those who prefer to exchange supplements by postal mail, we do not collect these measurements.

**If funding allows

	Baseline	6 maanden	12 maanden
Stolbuis (t.b.v. biobank serum)	2 x 8.5 ml	2 x 8.5 ml	2 x 8.5 ml
Trace element serum buis (t.b.v. biobank serum)	1 x 6 ml	1 x 6 ml	1 x 6 ml
EDTA buis (t.b.v. hematologie)	1 x 2 ml	1 x 2 ml	1 x 2 ml
EDTA buis (t.b.v. biobank plasma)	1 x 6 ml	1 x 6 ml	1 x 6 ml
EDTA buis (t.b.v. DNA isolatie)	1 x 6 ml	1 x 6 ml	1 x 6 ml
Totaal	**37 ml**	**37 ml**	**37 ml**

8.4 Withdrawal of individual subjects

Participants can leave the study at any time for any reason if they wish to do so without any consequences. Also, the investigator can decide to withdraw a participant from the study for urgent medical reasons.

In order to improve compliance during the intervention period regular protocols will be followed concerning missed behavioural therapy sessions by participant. This standard protocol includes active approach by the psychologist in case of no show.

8.5 Follow-up of subjects withdrawn from treatment

Participants who withdraw from the intervention (multi-nutrient pill/placebo or behavioural therapy) will be asked the reason(s) for drop out and they are motivated to continue with the measurements with the purpose to minimize loss of follow-up data and to make the intention to treat analysis and per-protocol analysis possible. However, the estimated drop out is already incorporated in the sample size calculation.

9. SAFTY REPORTING

9.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

9.2 AE's SAEs and SUSARs

9.2.1 Adverse Events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product / the experimental intervention. All adverse events reported spontaneously by the participant or observed by the investigator or his staff will be recorded. In the case of moderate Mild depression the participant will be requested to return for an extra follow-up assessment one month later where the depression status will be revaluated. A worsening on symptoms will be reported to the GP and the participant will be informed. If a participant develops severe depression or has suicidal thoughts/plans the GP will be inform by phone, and the participant will be advised to contact his/her GP.

9.2.2 Serious Adverse Events (SAEs)

A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

As the study concerns participants with sub-threshold depression it is possible that some participants may have a risk of suicide. A suicide protocol is included. If a participant is actively suicidal we shall contact the local psychiatrist/ doctor/ crisis team/ etc. and tell the participant that immediate help will be obtained.

All SAEs will be reported to the accredited METC that approved the protocol, according to the requirements of that METC. All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse events.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are Suspected Unexpected Serious Adverse Reaction (SUSARs) if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
    - Summary of Product Characteristics (SPC) for an authorised medicinal product;
    - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

Not applicable.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 Data Safety Monitoring Board (DSMB)

Not applicable

10. STATISTICAL ANALYSIS

Data from all for study centres will be pooled for the analysis. The main analyses will be based on intention-to-treat principles, but per-protocol analyses will also be conducted as a supplementary analysis. Baseline characteristics of the four intervention groups will be compared using chi-square tests, t-tests and analyses of (co)variance.

10.1 Primary study parameter(s)

The 2x2 factorial design is used to test whether the MooDFOOD nutritional intervention strategies (multinutrient supplements and FBC therapy) can prevent the onset of depression. The Kaplan–Meier method and log-rank test, and a Cox proportional hazards model will be used to analyze the time-to-event data (incidence of major depressive disorder (MDD)) and two-way and 4-way analyses will be performed to assess the separate and combined effects of our two intervention strategies. Adjustments will be made for the randomization strata country and history of depression. Additionally, mixed model regression analysis incorporating baseline, 3, 6 and 12 month assessments will also be used to test the main effects and interaction of the two intervention strategies on the onset of MDD and severity of depression symptoms (IDS-score). These mixed models takes into account the repeated nature of measurements within participants and the clustering due to country of participation.

10.2 Secondary study parameter(s)

To determine the impact of the two nutritional strategies and their interaction on the various secondary outcomes (food intake, BMI/body weight, physical activity, sedentary behaviour, PHQ-9 depression scores, anxiety severity, body image, emotional eating, cognitive restraint, uncontrolled eating and quality of life) mixed model regression analyses will be conducted. The models will allow for the clustering within countries.

10.3 Other analyses

Possible mediation effects due to changes in food-related behaviour, food intake, nutritional status (defined by serum cholesterol, carotenoids and fatty acids), body image, emotional eating, cognitive restraint, uncontrolled eating, body weight, physical activity and sedentary behaviour on the onset of depression and increase in depressive symptoms will also be analysed in (a 4 step) regression analysis with bootstrapping to assess the indirect effects of the mediation.[88,89]

A linear regression analysis will also be done in a subgroup of participants with blood samples available to evaluate the effect of change in nutritional status on the onset of

depression and change in depressive symptoms. This analysis will be adjusted for the type of intervention.

In order to establish whether the effectiveness of the nutritional strategies is similar or differential across countries, sex, education levels, income levels, baseline BMI level and age groups interaction terms will be generated and incorporated in Cox proportional regression analyses for the outcome incident MDD and in mixed models regression for depressive symptoms.

11. ETHICAL CONSIDERATIONS AND INSURANCE

11.1 Regulation Statement

The study will be conducted according to the principles of the Declaration of Helsinki (version of June 1964, last adopted 2013) and with the Medical Research Involving Human Subjects Act (WMO).

The study will employ an independent expert (Neeltje Batelaan, psychiatrist).

11.2 Recruitment and consent

Written information about the study will mailed to all potential participants during the mass mailing recruitment. Information will also be available on the website. Participants will have the opportunity to ask questions about the study during the telephone screening interview. Participants will be asked if they fully understand what participation in the prevention trial involves before signing the informed consent at the baseline assessment. The participants must also agree to allow us to contact their GP if the need arise (e.g. in case of suicidality), this is a compulsory requirement for participation in the study. For additional information participant may always can contact one of the investigators or the research assistant by phone.

11.2 Benefits and risks assessment, group relatedness

There will be no direct benefit for the subjects to participate in the study, although half of the participants will attend healthy eating behavioural therapy sessions which will hopefully improve their eating habits and thereby their well-being. The multi-nutrient supplements are designed to supplement dietary deficiency, the dosage of which will be similar to those available in over-the-counter supplements, hence well under the maximums according to the EFSA guidelines, thereby presenting no health risks to the participants. Additionally participants who are currently taking supplements containing the same vitamins/minerals will be requested to stop, thereby eliminating the risk of a double dose. Moreover, the levels of nutrients in the multi-nutrient supplements are such that even participants with naturally occurring hypernutrion (due to diet) will not exceed the maximum daily recommended dose. Participants who are taking supplements on prescription will be excluded from the study.

A blood draw will be down by a fieldworker or researcher who is qualified to draw blood thereby minimalizing the risk of injury. Furthermore. the blood draw is not a compulsory requirement in order to participate in the study, hence participants who do not like needles may abstain from this part of the trial.

During the screening process it is possible that some interested participants may indicate that they have suicidal thoughts. If this occurs whilst completing the on-line PHQ-9 questionnaire and automatic message will appear suggesting that the person contacts their GP. If this occurs during the telephone screening the potential participant will also be advised to contact their GP. The same applies for potential participants who are diagnosed as having a high depression score.

There is a potential risk that the participants, once enrolled, will develop suicidal tendencies. Additionally, once enrolled in the study we expect that some participants may become depressed. They will have achieved the study endpoint but will be allowed to continue in the study, if so desired. In order to ensure that these participants receive the correct medical help we will do the following:

- If the participant has a MINI diagnosis of MDD and **high** depressive symptoms (IDS score ≥ 39) and/or indications of suicidal thoughts/plans: inform GP by phone, and advice participant to contact his/her GP.

- If a participant is **actively suicidal**: contact the local psychiatrist/ doctor/ crisis team and tell participant that you will get him/her immediate help.

Apart from the risks named above, the participants will have the burden of remembering to take a pill and a capsule every day for a year and will have to make time to visit the study center at least 4 times. For those undergoing the behavioural therapy, this will involve 21 visits to the therapist in a period of one year. However, this study hopes to the effectiveness of nutritional supplements and behavioural therapy in the prevention of depression in an at risk population. If either of these therapies prove effective, this would be of great use to society, particularly those who are at risk.

11.3 Compensation for injury

The investigator has a liability insurance which is in accordance with article 7, subsection 9 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) the maximum claim per injury with a maximum of;
2. € 5.000.000,-- (i.e. five million Euro) per individual medical research, with the understanding that if the institution performs or has performed several scientific investigation the total sum insured is limited to € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.5 Incentives

Travel expenses for the baseline 3, 6, 9, and 12 months assessments will be reimbursed and participants will also receive a 10 euro VVV voucher at each of the four measurement waves. Furthermore, travel expenses for the FBC interventions will be reimbursed.

PROTOCOL FOR ASSESSING AND REPORTING DEPRESSION OR SUICIDALITY

The MoodFOOD research is a prevention trial but the project will include risk assessment for depression and suicidality. Any significant but not imminent risk should be reported to the General Practitioner of the participant and, if appropriate, other health care professionals, as soon as is reasonably possible according the following instructions:

If patients has a MINI diagnosis of MDD and an IDS-SR30 score higher or equal to 39 points (severe or very severe depression) and/or indications of suicidal thoughts/plans the MoodFOOD researcher will contact the GP by phone or email and advice participant to contact his/her GP.

If a participant is actively suicidal, as identified during the MINI psychiatric interview, the MoodFOOD researcher will contact the GP, psychiatrist or crisis team and will tell participant that will get him/her immediate help.

Participants with more than 13 points in the IDS score may continue in the MoodFOOD project, unless hospitalization is required.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

During the study, personal participant data (such as contact information, demographic variables and information concerning inclusion) collected from participants of MooDFOOD will be stored in an administrative/ electronic database at a VUmc server, which is secured with a password. Participants will be linked to an ID-number and identifiable information will be kept separate from the study data (collected with the questionnaires). Only the local research team at VUmc will have access to the code that connects the ID number to a person. Personal data will be stored for 15 years and will be destroyed after this time period. Participants will be registered with a six-figure code with which they can be identified in the database. The first three figures correspond to the individual patient, and the last to the country at which the patient received treatment. Anonymised data of all participating countries of the MooDFOOD study will be pooled for analyses by the data management team of GGZ inGeest.

A structured protocol will be developed for data delivery, aggregation and integration of all data collected at different international sites. These data will be cleaned centrally by the data management team of VUmc and delivered to researchers via safe data transfer methods. Quality control will be executed (out of range analysis, cross validation of variables, completeness of data) and a data catalogue will be developed for issuing of data.

12.2 Monitoring and Quality Assurance

The study will be conducted based on the quality handbook of the EMGO+. Direct monitoring of adherence to the EMGO+ guidelines is not applicable to this study. However, it is possible that the researchers will be audited. During an audit an independent researcher will investigate whether the study is being or has been done based on the quality standards of EMGO+.

12.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.3 Annual progress report

The investigators will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.4 End of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.5 Public disclosure and publication policy

The results will be submitted for publication to peer-reviewed scientific journals. In addition several periodic reports and one final report will be submitted to the European Commission representative, within 60 days after the end of the project (31/12/2018).This final report shall comprise:

- A final publishable summary report which includes: an executive summary, a summary description of project context and objectives, a description of the main trial results, the potential impact (including the socioeconomic impact of the project), and the main dissemination activities and exploitation of results/foregrounds;
- A plan for the use and dissemination of foreground, to spread awareness;
- A report covering the wider societal implications of the project, in the form of a questionnaire, including gender equality actions, ethical issues, efforts to involve other actors.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable

13.2 Synthesis

The supplements will be consumed in dosages which are widely available over the counter of local pharmacies and/or drugstores, and will contain dosages that adhere to dietary guidelines in all four participating countries. In order to prevent exceeding recommended daily doses, participants will be required to stop taking any other multi-vitamin supplements. Furthermore, participants who are required by their GP to take vitamin supplements will be excluded from the study, thereby also eliminating the risk of nutrient deficiency for those allocated a placebo pill. Hence we perceive that there will be no risks to participants.

Analysis of microbiological data will be provided by Ferrer International as soon as the batches are released by the corresponding Q.A.

The labelling of the product shall advice against use during pregnancy, breast feeding. Additionally advice shall be given not to use the supplements in the case of hypersensitivity and allergy.

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