RESEARCH PROTOCOL (September 2023)

"Diet quality of pregnant women with a strict plant-based diet versus an omnivorous diet: a focus on vitamin B12 intake and other nutritional and pregnancy-related outcomes."

PROTOCOL TITLE Diet quality of pregnant women with a strict plant-based diet versus an omnivorous diet: a focus on vitamin B12 intake and other nutritional and pregnancy-related outcomes.

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
AVG	**Algemene Verordering Gegevensbescherming**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IC	**Informed Consent**
KNOV	**Koninklijke Nederlandse Organisatie van Verloskundigen**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
MUMC+	**Maastricht University Medical Center+**
RIVM	**Rijksinstituut voor Volksgezondheid en Milieu**
(S)AE	**(Serious) Adverse Event**
Sponsor	**The sponsor is the party that commissions the organization or performance of the research, for example, a pharmaceutical company, academic hospital, scientific organization, 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 subsidizing party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
WHO	**World Health Organization**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: The number of people adhering to a strict plant-based diet is increasing globally. Adequate maternal nutrition during pregnancy is essential for fetal development, as deficiencies in macro- and micronutrients can cause maternal and neonatal complications. Nutrition can play a role in developing pre-eclampsia, premature birth, and intra-uterine growth restriction. Although there is evidence of nutrient deficiencies in people who follow a strict plant-based diet and the significance of maternal diet for both maternal and fetal outcomes, there has been limited research on the nutritional intake and outcomes of pregnant women on a strict plant-based diet. Additionally, to the best of our knowledge, very limited research has been published on the incidence of pregnancy-induced hypertension, pre-eclampsia, gestational diabetes, or pre-term birth in women on a strict plant-based diet. The relation between these outcomes and nutritional intake/status is also unknown in this specific group of women, even though the number of people with a strict plant-based diet is increasing. The only two studies that studied pregnancy-related outcomes in women on a strict plant-based diet were performed in Israel and were not powered to show statistical differences between a strict plant-based diet and obstetrical outcomes. Additionally, the dietary intake in Israel differs from other parts of the world. Thus, it is impossible to ascertain if the results also apply to a population with a distinct diet. Recently, the Royal Dutch Organization of Midwives (Koninklijke Nederlandse Organisatie van Verloskundigen, KNOV) published 'The Guide for Vegetarian and Vegan Diet in Pregnancy.' They acknowledge the limited availability of scientific research concerning the strict plant-based diet in pregnancy and the non-existence of data on Dutch pregnant women. They recommend performing additional blood tests for vitamin B12 and D in case of possible nutrition deficiencies in this group of pregnant women. A significantly lower ferritin level is also described in pregnant women on a strict plant-based diet. In conclusion, even though the nutritional intake during pregnancy is vital for both mother and child, the nutritional intake amongst Dutch women on a strict plant-based diet during pregnancy is unknown. Previously published results from Israeli women do not apply to the Dutch population, as nutritional intake differs between countries. The recently published KNOV guide suggests studying vitamin blood levels during pregnancy. To advise and update guidelines for Dutch women on a strict plant-based diet during pregnancy, additional research on nutritional intake and the nutritional status of Dutch women on a strict plant-based diet and to compare this with the nutritional status of omnivorous pregnant women is crucial.

Objective:

The primary objective of the study is to compare vitamin B12 intake between pregnant women with a strict plant-based diet and pregnant women with an omnivorous diet. The secondary aim of this study is to compare the nutritional intake and status of pregnant

women following a strict plant-based diet with those following an omnivorous diet. Additionally, the maternal and fetal outcomes of pregnant women on a strict plant-based diet will be compared to those on an omnivorous diet.

Study design: The study comprises an observational non-inferiority design.

Study population: Pregnant women with a strict plant-based diet (n=128) and pregnant women with an omnivorous diet (n=128) will be recruited to participate in this study.

Main study parameters/endpoints: The main study parameter is the vitamin B12 intake (mcg/day). Additional parameters are the daily intake of other macronutrients and micronutrients and the adherence to Dutch dietary guidelines, plasma vitamin D, plasma vitamin B12, plasma hemoglobin, and plasma ferritin. Other parameters are maternal and fetal outcomes (e.g., gestational age at delivery, gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, weight gain during pregnancy, birth weight, and breastfeeding initiation).

Nature and extent of the burden and risks associated with participation, benefit, and group relatedness: Participants may encounter a minor burden during this study. The participant will complete a health-related questionnaire. The estimated time required to complete the questionnaire is approximately 10 minutes. Additionally, between 27 and 30 weeks of pregnancy, participants will fill out a three-day food diary. It is estimated that the questionnaire will take approximately 30 minutes to complete each day. Finally, the participants must complete a questionnaire following the baby's delivery. This questionnaire will cover various aspects, such as the pregnancy, delivery, and blood results obtained during pregnancy. The estimated time required to complete the questionnaire is approximately 15 minutes.

During the blood sampling, 10 ml of blood will be collected using an evacuated tube system through venepuncture. This may cause minor discomfort and potentially result in a small, temporary hematoma at the puncture site. Taking blood samples is a routine part of clinical practice, not solely for this study. Pregnant women typically undergo venipuncture between 27 and 30 weeks as part of regular obstetrical care. Participants can have their blood sampled at a nearby laboratory without traveling for the study.

The study's results will provide valuable information about the nutritional intake and status of pregnant women who follow a strict plant-based diet compared to those who follow an omnivorous diet during pregnancy. It will also examine the impact of these diets on the health of both the mother and fetus. This information can be used in developing and improving guidelines on diet in pregnancy and may contribute to the health of pregnant women and their newborn children in the future.

1. INTRODUCTION AND RATIONALE

The number of people adhering to a strict plant-based diet, which consists of fruits, vegetables, grains, legumes, nuts, seeds, herbs, and spices, and excludes all animal products (often also referred to as a vegan diet), is increasing globally. The prevalence is estimated to be 1.5% of the Dutch population, and the number of vegans in Great Britain quadrupled between 2014 and 2019 to 1.21% of the population.[1]

Adequate maternal nutrition during pregnancy is essential for fetal development, as deficiencies in macro- and micronutrients can cause maternal and neonatal complications. Nutrition can play a role in developing pre-eclampsia, premature birth, and intra-uterine growth restriction. Vitamin B12, folate, iodine, calcium, zinc, selenium, magnesium, and iron are essential elements in maternal and fetal health.[2] In a strict plant-based diet, sufficient intake of vitamin B12 can only be achieved by using supplements since vitamin B12 is naturally not present in plant-based foods unless being fortified. Maternal iron deficiency, linked to inadequate ingestion of iron, but also of, e.g., vitamin B12 or folate, in the first and second trimester, can lead to premature birth or low birth weight and jeopardize the newborn's health.[3,4] Iron deficiency is associated with neuronal changes and problems in myelinization, neuronal transmission, and frontal cortex and basal ganglia development.[5] Anaemia is related to a higher maternal mortality rate and can lead to miscarriage during the first trimester.[5,6] Maternal iodine deficiency is associated with aberrations in the neuronal development of children. It can cause irreversible damage to the central nervous system, instilling permanent mental retardation.[7] Calcium deficiency increases the risk of maternal pre-eclampsia and is medically induced prematurely.[8] Additionally, it can contribute to spontaneous prematurity and intra-uterine growth restriction, and it implies a variety of physical and cognitive consequences in the long term, including increased atrophy.[9] Selenium deficiency can cause alterations in the lipid profile, mental and psychomotor delay, oxidative stress in the mother and the fetus, premature birth, miscarriage, and problems in the neural tube of the newborn.[2] Selenium acquired during pregnancy is maintained in newborns' reserves; the build-up of this mineral in the uterus occurs in the third trimester.[2] When in low concentrations in newborns, especially prematurely, they are at higher risk for developing retinopathy, bronchopulmonary dysplasia, and other lung disorders. However, excess selenium can trigger cardiovascular problems, dyslipidaemias, and insulin resistance.[2] Zinc deficiency during pregnancy can cause premature birth, complications in childbirth, and neurological deficits. It can also cause a reduced natural skin and mucosa barrier function and deleterious effects on the immune system.[2] Magnesium deficiency can lead to severe pre-eclampsia, premature birth, and hypoxic-ischemic encephalopathy.[10,11] Maternal intake of macronutrients is also of importance for both mother and fetus. Maternal energy intake is needed to provide energy for the fetus for the synthesis of new tissue;

protein is involved in both structural and functional biological roles and influences birthweight; and fatty acids impact the development of the brain and retina in the fetus and the risk of preeclampsia.[12] Although evidence is limited, a low glycaemic index, low glycaemic load, and a high fiber diet may benefit preconception or early pregnancy for women at risk of developing gestational diabetes mellitus or preeclampsia or having a large for gestational age infant.[12]

In a systematic review by Bakalouhi *et al*., including 48 studies (12 prospective cohorts and 36 cross-sectional studies), differences in the nutrient intake and nutrient status of non-pregnant people on a strict plant-based diet compared to World Health Organization recommendations were summarised.[13] Sample sizes differed between 6 and 2596 participants. The general conclusions of the review were that people on a strict plant-based diet generally have a higher fiber intake and a total energy intake from fat comparable to an omnivorous diet. However, deficiencies or low intake of various nutrients were also described. Recommended levels of protein intake (i.e., 15% of daily energy intake, 0.66 g/kg daily) were not always reached. The vitamin B12 intake was significantly lower, and a serum deficiency of vitamin B12 was frequently observed. Even though the iron intake was higher among people on a strict plant-based diet compared to individuals who followed other dietary patterns, this did not always translate into higher ferritin levels due to the lower bioavailability of non-haem iron from plant-based foods. However, overall, the risk of iron deficiency among vegan women was not higher than in omnivorous women. Vitamin D intake was also found to be lower than the WHO limit, but deficiencies were only sometimes observed. Calcium intake was low due to the exclusion of dairy products and the lower bioavailability of calcium in plant-based foods, but still above the WHO limit of 525 mg/day. Zinc intake was reduced among people on a strict plant-based diet, and a risk of deficiency was frequently observed. Intake of iodine below the limit was reported for most people on a strict plant-based diet in several studies. And lastly, there seemed to be a decreased selenium intake. Importantly, in the last ten years, more and more meat substitutes and other plant-based products have become available on the market. Therefore, the current dietary intake will differ from the nutritional intake 20 or 30 years ago.

Despite the observed findings on deficiencies in nutrient intake in the general population on a strict plant-based diet and the importance of maternal diet for maternal and fetal outcomes, limited research has been performed on the nutritional intake and the maternal and fetal outcomes of pregnant women on a strict plant-based diet. Additionally, to the best of our knowledge, very limited research has been published on the incidence of pregnancy-induced hypertension, pre-eclampsia, gestational diabetes, or pre-term birth in women on a strict plant-based diet. The relation between these outcomes and nutritional intake/status is also unknown in this specific group of women, even though their number is

increasing. The only two studies that researched pregnancy-related outcomes in women on a strict plant-based diet were performed in Israel and were not powered to show statistical differences between a strict plant-based diet and obstetrical outcomes. The prospective observational study by Avnon et al. among 60 pregnant women on a strict plant-based diet compared to other diets showed an increased risk of small-for-gestational-age newborns compared to omnivores (RR 5.9, 95% CI 1.2-21.8) with a lower birthweight of newborns from mothers with a strict plant-based diet compared to omnivores (3015 gram ± 420 g versus 3328 gram ± 495 g, p<0.001).[14] The most recent study by Kesary et al. included 234 women on a strict plant-based diet in a retrospective, web-based study and compared them with vegetarians and omnivores.[15] After adjusting for BMI, they concluded that there was no association with lower birth weight for offspring of women on a plant-based diet during pregnancy compared to omnivores, with an adjusted odds ratio for small for gestational age of 1.59 (95% CI 0.95 – 2,65). There was a decreased risk for gestational diabetes, although this association did not reach significance (OR = 0.54 95% CI 0.28-1.03). A strict plant-based diet had no significant association with the risk of preterm birth. The dietary intake in Israel, where both studies were performed, differs from other parts of the world.[16] It is impossible to determine whether the results apply to a population with a different diet.

Recently, the Royal Dutch Organization of Midwives (Koninklijke Nederlandse Organisatie van Verloskundigen, KNOV) published ‘the guide for vegetarian and vegan diet in pregnancy.’[19] They acknowledge the limited availability of scientific research concerning the strict plant-based diet in pregnancy and the non-existence of data on Dutch pregnant women. Therefore, they recommend additional blood tests for vitamin B12 and D in case of possible nutrition deficiencies in this group of pregnant women. A significantly lower ferritin level is also described in pregnant women on a strict plant-based diet.

In conclusion, even though the nutritional intake during pregnancy is vital for both mother and child, the nutritional intake amongst Dutch women on a strict plant-based diet during pregnancy is unknown. Previously published results from Israeli women do not apply to the Dutch population as nutritional intake differs between countries. The recently published KNOV guide suggests checking vitamin blood levels during pregnancy. To advise and update guidelines for Dutch women on a strict plant-based diet during pregnancy, additional research is crucial on nutritional intake and the nutritional status of Dutch women on a strict plant-based diet.

This study will primarily evaluate nutritional intake of vitamin B12 of pregnant women on a strict plant-based diet, as this nutrient needs to be supplemented for these women to have a sufficient vitamin B12 intake. Secondly, the study will assess the nutritional intake of other relevant micronutrients and macronutrients, the nutritional status (vitamin B12, D, ferritin, and hemoglobin), and their maternal and fetal outcomes.

2. OBJECTIVES

2.1 Primary Objective

In this observational non-inferiority study, we aim to compare vitamin B12 intake in pregnant women with a strict plant-based diet and pregnant women with an omnivorous diet. We hypothesize that the vitamin B12 intake amongst women with a strict plant-based diet is non-inferior to pregnant women with an omnivorous diet.

2.2 Secondary Objectives:

The secondary aim of this study is to compare pregnant women with a strict plant-based diet and pregnant women with an omnivorous diet concerning their:

- Intake of micronutrients and macronutrients
- Plasma vitamin B12, vitamin D, ferritin, and hemoglobin
- Maternal and fetal outcomes, including gestational age at delivery, gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, weight gain during pregnancy, and fetal birth weight.

3. STUDY DESIGN

In this observational non-inferiority study, a total of 256 pregnant women will participate, with n=128 pregnant women with a strict plant-based diet and n=128 pregnant women with an omnivorous diet.

Participant recruitment will start after the Medical Research Ethics Committee (METC) of Maastricht University Medical Center+ (MUMC+) has approved this study according to the revised version of the Guidelines of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013).

Participants will be recruited via their obstetrician (midwife or gynecologist). They will provide a brief explanation to potentially eligible pregnant women regarding the study. Furthermore, participants will be recruited through nutrition and pregnancy-related social media advertisements and other online platforms (website/newsletter Nederlandse Vereniging voor Veganisme, nutrition and/or pregnancy-related magazines).

If a woman shows interest, she will be directed to the study website (www.voedingsonderzoek.nl), which contains concise information about the study (*see "E3_Advertentieteksten_NL…._Versie1.0_19-07-2023")*. Potential participants can contact the researchers at the Maastricht University Medical Center+ via e-mail. In addition, we will be contacting women who participated in another previously conducted study that has been

finished already by our research group titled "Raising Children on a Vegan Diet: A Qualitative Exploration of Feeding Practices and Determinants of Mothers Following a Vegan Diet." These women have given their consent to be approached for future research and will be contacted via email (*see "E3_WervingstekstInterviewstudie_NL…_V1.0_19.07.2019"*). The researcher will contact those who respond and invite them to fill out a screening questionnaire about their nutritional diet, pregnancy, and medical history (*see "F1_vragenlijstscreening_NL..._Versie1.0_19-07-2023")*. The researcher will also send the participant information brochure (PIF) through email or regular mail.
The PIF and the informed consent form are enclosed in this study protocol ("*see E1+E2_Proefpersoneninformatie_NL……Versie1.0_datum"*). When considered eligible to participate in the study, the participant will be asked to sign informed consent.

Women are given at least three days to decide if they want to participate in the study. If the woman is interested and willing to participate, an informed consent form will be signed (co-signed by the investigator). The signing of the informed consent form needs to be finished before 27 weeks of pregnancy for a participant to join the study. After signing informed consent, the total duration of the study will be from 27 weeks of pregnancy until the post-partum questionnaire is received. A total of 256 pregnant women will be included in the study.

The study consists of the following:

1. General health-related questionnaire
2. 3-Day food record
3. Blood sample
4. Questionnaire regarding pregnancy, delivery, and post-partum period

These steps are briefly described below; more elaborate information can be found in paragraph 5.2.

3.1 General health-related questionnaire

Once the participant has provided their informed consent, they will receive an email containing a link to a general health-related questionnaire which they will need to complete (*see "F2_vragenlijstgezondheid_NL..._Versie1.0_19.07.2023")*. This email will be sent via Castor.

3.2 3-Day food record

As part of standard care in the Netherlands, pregnant women have their blood sampled between 27-30 weeks. For this study, participants will be asked to keep track of their

nutritional intake for three days, including two weekdays and one weekend day, in the two weeks leading up to their blood test. This will help establish a connection between their blood results and dietary habits. To log their daily intake, participants will be directed to download the 'Traqq' app (*see "F3_traqqapp_NL..._Versie1.0_04.08.2023")*

3.3 Blood sample

The participant will be asked to provide their obstetrician at a regular consultation before 27 weeks of pregnancy with an information document about the blood sampling (see *"E4_overigvoorlichtingsmateriaal_NL…_Versie1.0_21-07-2023*"). In this document, the obstetrician is requested to measure hemoglobin, ferritin, vitamin B12, and vitamin D in the blood sample taken from the participant as regular pregnancy care between 27-30 weeks of pregnancy. The obstetrician most likely already conduct these blood tests as part of their routine care. If this is the case, there's no need for any extra tests to be done. The obstetrician can proceed with their usual actions of care based on the study results; no additional study-related actions are required.

3.4 Questionnaire pregnancy and post-partum period

Six weeks after the participants' due date, they will be asked to complete the questionnaire regarding their pregnancy, delivery, and postpartum period (*see "F4_vragenlijstpstpartum_NL..._Versie1.0_19.07.2023")*. They will receive the link to this questionnaire by email, sent via Castor.

4. STUDY POPULATION

4.1 Population (base)

Pregnant women with a strict plant-based diet from the Netherlands and pregnant women with an omnivorous diet will be recruited in the Netherlands.

4.2 Inclusion criteria

To be eligible to participate in this study, a participant must meet all of the following criteria.

- Before 27 weeks of pregnancy
- Age >18 years
- Strict plant-based diet (vegan diet), defined as a diet without ingestion of animal products (i.e., intake less than once a month during) the entire pregnancy, or omnivorous diet, defined as a diet that includes meat
- Willing and able to give written informed consent and to understand, participate and comply with the research project requirements

4.3 Exclusion criteria

A potential participant who meets any of the following criteria will be excluded from participation in this study:

- Food allergy that influences the nutritional intake, e.g., milk allergy, celiac disease, and lactose intolerance
- Medical history of gastrointestinal diseases such as Crohn’s disease and other diseases associated with vitamin B12 deficiencies such as (pre-existing) diabetes.
- Medical history of abdominal surgery interfering with nutrient uptake, such as gastric bypass and intestinal resections
- Pre-existing cardio-vascular diseases or auto-immune diseases
- Insufficient understanding of the Dutch language

4.4 Sample size calculation

Our calculation for the sample size is based on the primary outcome measure, which is the intake of vitamin B12. Pregnant women in the Netherlands are advised to consume 3.3 mcg of vitamin B12 daily. According to the 'Voedselconsumptiepeiling' of the RIVM (Rijksinstituut voor Volksgezondheid en Milieu) conducted between 2012-2016, 63.2% of Dutch women (without considering their diet) had a sufficient intake.[17] The significance level (alpha) and, thus, the chance of a false positive result will be 0.05. To obtain a power (1-beta) of 80%, with a percentage of ‘success’ of 63.2% in the group of women with a strict plant-based diet

and the group of women with the omnivorous diet, and a non-inferiority limit of 15%, a sample size of 128 participants per group is required. So, a total sample size of 256 participants is required.
There will only be recruitment for additional participants if the primary outcome (vitamin B12 intake) is missing. Sample size calculation was conducted on the statistical power analysis website https://www.sealedenvelope.com/power/binary-noninferior/.

5. METHODS

5.1 Study parameters/endpoints

5.1.1 Main study parameter/endpoint

The primary outcome is vitamin B12 intake in mcg/day.

5.1.2 Secondary study parameters/endpoints

1. Nutritional intake (micronutrients and macronutrients)
2. Plasma vitamin B12, vitamin D, ferritin, and hemoglobin
3. Maternal and fetal outcomes: gestational age at delivery, gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, gestational weight gain, birthweight

5.1.3 Other study parameters

Age, parity, Body Mass Index before pregnancy (BMI) and after pregnancy, smoking, alcohol use, drug use, educational level, employment, medical history, obstetrical history, family history (e.g. (gestational) diabetes, pregnancy-induced hypertension, pre-eclampsia), medication use, supplement use, when they started being on the strict plant-based diet

5.2 Study procedures

Questionnaires

All questionnaires will be completed in a secured, electronic environment (Castor) that can be accessed via the internet by electronic devices (telephone, tablet, or computer).

Participants will be asked to fill out the following questionnaires

- Health-related questionnaire
  See "F2…Naam_studienummer_Versie1.0_datum"
  This questionnaire comprises 30 questions covering various topics and takes about 15 minutes to complete. The participants are requested to provide their birth details, educational qualifications, employment status, and dietary habits during pregnancy. In addition, they are inquired about their pregnancy, obstetric record, present height, and weight, smoking habits, alcohol consumption, drug usage, medication intake, and supplement usage.
- Three-day food records
  see "F3_traqqapp_NL..._Versie1.0_04.08.2023"

All participants will receive instructions to use the Traqq app (developed by Wageningen University) to record their food intake for three days. This will include two regular days and one weekend day. The food records will contain information about the time of day when the food was consumed and the type and amount of food eaten. Participants will receive instructions on estimating serving sizes and recording recipes in the app. The food record must be completed within the two weeks before the blood sample collection between 27-30 weeks of pregnancy.

- Maternal and fetal outcomes questionnaire
  See "F4…Naam_studienummer_Versie1.0_datum"
  This questionnaire comprises a maximum of 15 questions covering various topics and takes about 10 minutes to complete. Participants are asked about, e.g., their blood sample results, the occurrence of complications related to pregnancy, the delivery, and the newborn. Participants will have access to the questionnaire six weeks after their anticipated due date, and must be completed within 4 weeks.

Blood sampling

The local laboratory will collect blood samples from an antecubital vein in the forearm, which may cause minor discomfort and result in small and temporary hematoma or bruises. However, it's important to note that obtaining blood samples from pregnant women for hemoglobin testing is a routine part of clinical practice between 27 and 30 weeks of pregnancy, and not specifically for this study. For study purposes, 10mL of additional blood will be sampled per participant during this blood withdrawal.

5.3 Withdrawal of participants

Participants can leave the study at any time for any reason if they wish to do so without any consequences.

5.4 Replacement of individual participants after withdrawal

Participants will not be replaced after signing informed consent. However, if the primary outcome (vitamin B12 intake in mcg/day) is missing, an additional participant will be recruited to meet the calculated sample size.

5.5 Premature termination of the study

There are no foreseeable reasons for the study to end prematurely.

6. SAFETY REPORTING

6.1 Temporary halt for reasons of participant safety

In accordance with section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize the subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt, including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

6.2 AEs, SAEs and SUSARs

The study makes use of questionnaires and food diaries. Blood sampling is part of current and daily clinical routine; for this study, additional blood will be sampled (10mL). Therefore, study-related adverse events (AEs), serious adverse events (SAEs), and suspected unexpected serious adverse reactions (SUSARs) are not applicable.

7. STATISTICAL ANALYSIS

- All data will be presented quantitatively. Means and standard deviations or medians with inter quartile ranges depending on variable distributions of maternal and fetal outcomes will be given for continuous variables (such as gestational weight gain and birthweight), and frequencies and percentages will be presented for categorical variables (such as gestational age at delivery, gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, pre-term birth).
- Continuous (such as (micro)nutrient intake and blood levels, age, BMI) will be presented as means with standard deviations (normal distribution) or median with interquartile range (non-normal distribution) and categorical (such as smoking, alcohol use, drug use, education, employment, obstetrical history, obstetrical family history, medication use, supplement use) will be presented as percentages.
- Comparison between women on a strict plant-based diet and the omnivorous group will be presented with the independent t-tests (normal distribution) or Mann-Whitney U test (non-normal distribution), or chi-square test (for categorical variables (with Fisher exact when needed)). Additionally, a multivariable linear regression analysis will assess the association between maternal and fetal outcomes with vitamin B12 intake, adjusted for potential confounders (such as BMI).

7.1 Primary study parameter

The main study parameter is vitamin B12 intake (mcg/day).

7.2 Secondary study parameters

Secondary study parameters are:

- Other micro- and macronutrient intake
- Plasma vitamin B12, vitamin D, ferritin, and hemoglobin
- Gestational age at delivery, gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, gestational weight gain, birthweight, initiation of breastfeeding

8. ETHICAL CONSIDERATIONS

8.1 Regulation statement

This study has to be approved by the Medical Ethical Committee of Maastricht University (METC). The study will be conducted according to the Declaration of Helsinki (8th revision, 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

8.2 Recruitment and consent

After approval of the study by the METC of MUMC+, participants will be recruited via their obstetrical healthcare giver (midwife or Gynaecologist). The obstetrician will provide pregnant women with a brief introduction to the study. If the woman expresses interest, she can be asked if the researcher may contact her to give more information about the study orally. The obstetrician will then share the woman's contact details with the researcher. The woman can visit the study website (www.voedingzwangerschap.nl) to find brief information about the study and email the researchers at Maastricht University Medical Center+.

Furthermore, participants will be recruited through nutrition and pregnancy-related social media advertisements and other online platforms (website/newsletter Nederlandse Vereniging voor Veganisme, nutrition and/or pregnancy-related magazines).
Lastly, women who participated in the study ‘Raising children on a vegan diet, a qualitative exploration of feeding practices and determinants of mothers following a vegan diet’ and who gave consent to be approached for further research will be contacted by email or letter (*see “E3_WervingstekstInterviewstudie_NL…_V1.0_19.07.2019”*). Women will be referred to the study website (www.voedingzwangerschap.nl), where brief information about the study can be found and where she can contact the researchers at the Maastricht University Medical Center+ via e-mail.

If a woman is interested in participating in this study, she can contact the research team by e-mail or telephone, as shown on the website. Women can always contact the researcher or independent medical doctor if any question arises and will be referred to the VWO brochure “General information for research participants,” which contains general information about medical-scientific research. The researcher will contact the women interested in the study by e-mail or telephone and send them the PIF via e-mail or general mail (“*see E1+E2_Proefpersoneninformatie_NL……Versie1.0_datum”*). Participation will be voluntary. Three days (minimum) until 27 weeks of pregnancy (maximum) are provided to decide whether the woman would like to participate. The woman is asked to contact the investigator by email or telephone if she wants to participate. However, if the participant does not contact

the researcher, the researcher will contact the participant (at the earliest three days after sending the written information brochure). Permission to contact the patient will be logged in the subject screening and enrolment log. If the woman is interested and willing to participate, she will be asked to sign a digital informed consent form that will be co-signed by the investigator, both in duplicate. The informed consent form has to be filled out completely. The date of the assignment is necessary.

The participants' privacy will be protected, meaning the study results will be related to an anonymous identification code. Codes and related participant information will not be available to individuals other than the principal investigator and a researcher (Drs. D. Meulenbroeks). They will be stored in a password-protected file. Participants can appeal to the independent doctor (Dr. R. van Golde) appointed to this study at any time. Participants will be informed that their decision to participate is voluntary, and they can withdraw at any time without giving a reason. Participants can be informed about the group results at the end of the study.

8.3 Benefits and risks assessment, group relatedness

Participants will not receive any direct benefits from participating in this study. The results of this study will be used to gain more knowledge about nutritional intake by pregnant women in the Netherlands with a strict plant-based diet and an omnivorous diet, which can affect the health of the mother and the unborn baby. There are no specific risks that need to be considered.

8.4 Compensation for Injury

We will apply for a waiver for the insurance because of the nature of this study. Liability insurance is available for current study.

8.5 Incentives

To thank the participants for participating, a pregnancy or newborn photoshoot will be made available to win for every 50 participants who join the study.

9. ADMINISTRATIVE ASPECTS, MONITORING, AND PUBLICATION

9.1 Handling and storage of data and documents

Data will be handled confidentially (coded), and the privacy of the participants will be guaranteed, according to the Dutch Personal Data Protection Act (“Algemene Verordering Gegevensbescherming” (AVG)). All eCRFs (in Castor) will be coded so that no personal information about the participant can be derived.

The project team members, assigned monitors from the CTCM, IGZ, and members of the medical ethical committee will have access to the research data. All primary documents and data shall be kept for 15 years after the end of the experimental phase of the study for possible inspection. The electronic CRF, electronic diary, and electronic questionnaires (using Castor) will be developed, considering current regulations.

9.2 Monitoring and Quality Assurance

The Clinical Trial Centre Maastricht will perform monitoring and quality assurance.

9.3 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

9.4 Annual progress report

The sponsor/investigator will submit a summary of the trial's progress to the accredited METC once a year. Information will be provided on the date of inclusion of the first participant, the number of participants included, and number of participants that have completed the trial, problems, and amendments.

9.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within eight weeks. The end of the study is defined as the last patient’s post-partum information received or six months after the last participant gave birth.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason for such an action. If the study ends prematurely, the sponsor will notify the accredited METC within 15 days, including the grounds for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final

study report with the study's results, including any publications/abstracts, to the accredited METC.

9.6 Public disclosure and publication policy

Publication policy is in agreement with the Centrale Commissie Mensgebonden Onderzoek (CCMO-statement publicatie beleid). The results of the scientific research will be published in peer-reviewed scientific journals. Both positive and negative results of the study will be disclosed. The article's authorship shall be determined in appropriate consultation based on a considerable contribution to the set-up and execution of the study and active participation in publication.

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