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Executive Summary

This is a report on the results of the baseline dietary assessment conducted in Study I-Phase I (Observational Phase) in children and in Study II-Baseline Phase in lactating mothers (including Donors providing the Human Milk). In children diet was evaluated with a Food Frequency Questionnaire, a 4-day food record and a questionnaire assessing diet quality (Mediterranean diet adherence), while in lactating mothers dietary intake was assessed with a Food Frequency Questionnaire and a 24-h hour recall. Results are presented by study and by the different study groups.

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Definitions, Acronyms and Abbreviations

Acronym	Title
BMI	Body Mass Index
CEN	Complete enteral nutrition
DHM	Donor Human Milk
FFQ	Food Frequency Questionnaire
FT-OMM	full term infants receiving Own Mother's Milk
HM	Human Milk
OMM	Own Mother's Milk
PT-DHM	Pre-term infants fed with pasteurized Donor Human Milk
PT-OMM	Pre-term infants fed with Own Mother's Milk
RBW	Recover of Birth Weight

1. Preamble

Two clinical studies, namely Study I and Study II are implemented within NUTRISHIELD, each one aiming to produce input regarding different aspects of the project. The current deliverable presents the results of the **baseline dietary assessment** conducted in Study I: Personalised nutrition against obesity/diabetes for children (Phase I), and in Study II: Personalised nutrition for lactating mothers– (Baseline Phase), in children and lactating mothers, respectively. Study III: (Personalised nutrition against cognitive decline for children), is part of a trial in progress and is implemented outside NUTRISHIELD project with dietary assessment being held by Donders Institute. Thus, the current deliverable focuses on data from Studies I & II.

1.1. Objective of the deliverable

The objective of the deliverable D5.1 is to provide the results that emerged from the baseline dietary assessment conducted in the context of Studies I & II. Energy and nutrients' intake, consumption of foods of specific food groups and adherence to the Mediterranean diet is presented for children and lactating mothers, based on the different tools implemented within each Study. In specific, in Study I, children's dietary intake was evaluated with a Food Frequency Questionnaire (FFQ), a 4-day food record and the KIDMED questionnaire, whereas in Study II, lactating mothers' dietary intake was assessed with a FFQ and one 24-h recall.

1.2. Content of the deliverable

The Deliverable D5.1 includes:

- Dietary Assessment of children in Study I-Phase I (namely, the Observational phase)

- Dietary Assessment of lactating mothers (including Donors providing the Human Milk) in Study II-Baseline Phase (namely, the baseline and the 1st month of the Observational phase).

2. Dietary Assessment of children in Study I-Phase I

2.1. Short description of the study protocol

Study I is a two-arm, parallel, randomized controlled study, with a dietary intervention (Phase II) for children (8-18 years old) with obesity or diabetes (type I or II), with an Observational Phase (Phase I) preceding. It is being carried out in Ospedale San Raffaele hospital in Italy, between M23 and M43. The study protocol has been approved by the Ethics Committee, and written informed consent is obtained from all parents/caregivers of included participants.

The study is divided into two phases:

Phase I: the Observational phase, lasting from M23 to M34. During this period children aged 8-18 years old with obesity, diabetes, or healthy children were recruited and their diet and biomarkers were assessed once. At the end of this phase, the study has paused for four months (from M35 to M38) in order to perform machine learning analyses, feed the platform and train the personalized nutritional algorithm with the results from the preceding assessments of the Observational Phase.

Phase II: the Intervention phase, lasting six months. During this period children with obesity and/or diabetes will be randomly allocated to one of the following groups:

- Group A (Intervention) will receive a NUTRISHIELD-derived personalized diet
- Group B (Control) will follow the standard European dietary guidelines for obesity/diabetes, i.e. the usual care/control diet

Diet, biomarkers and clinical data will be evaluated in the beginning, during and in the end of the intervention phase.

The current deliverable focuses on dietary data collected from Phase I (i.e. Observational Phase). In detail, three methods of dietary assessment were employed during Phase I, namely a 4-day food record completed in a mobile app with food pictures uploading, a FFQ and a short questionnaire assessing diet quality (KIDMED). The rationale of using both a FFQ and a 4-day food record was to cross-match intake data, and the dietary assessment modality proved to be the most valid to be used in Phase II of the study. Finally, the KIDMED was used as a quick index of diet quality. All the dietary assessment tools are thoroughly described below.

Regarding the 4-day food record, all children with the help of their caregivers (for ages <9 years) were asked to record all foods and drinks consumed for four days in the NUTRISHIELD app and upload pictures of all the food and drinks consumed. Detailed instructions were incorporated in the app, explaining how dietary intake should be recorded as well as instructions on how the pictures should be taken. For more details on this, please see Deliverable 6.4. However, as the NUTRISHIELD app was not ready at the beginning of the study (the app was released in M32), and in order to obtain data from the participants recruited before this period, it was proposed to complete the record in paper form, till the app is released. Nutrients' intake was calculated using a standard food analysis program [the Nutritionist Pro™ Diet Analysis software (Axxya Systems, Woodinville, WA, USA)].

The second method of dietary assessment was the implementation of a semi-quantitative FFQ. This questionnaire comprises 69 questions on the consumption of foods that are commonly eaten by various populations throughout a year, including dairy products, cereals, fruits, vegetables, meat, fish, legumes, added fats, alcoholic beverages, stimulants and sweets [1]. Using a 6-grade scale (“never/rarely”, “1-3 times/month”, “1-2 times/week”, “3-6 times/week”, “1 times/day”, “≥2

times/day”), participants were required to indicate the absolute frequency of consuming a certain amount of food, expressed in g, milliliters or other common measures, such as slice, tablespoon or cup, depending on the food. The previous month was set as the timeframe. The FFQ was completed by the children with the help of the caregiver when needed. From the answers to the FFQ, total energy intake, and intake of macro-nutrients were calculated.

Diet quality was assessed by the KIDMED questionnaire. The KIDMED was originally developed in an attempt to combine the Mediterranean diet characteristics as well as the general dietary guidelines for children in a single index [2]; it is based on the principles for sustaining a healthy, Mediterranean-type pattern (e.g. daily fruit and vegetable consumption, weekly fish and legumes intake), as well as on those that undermine it (e.g. frequent fast-food intake, increased consumption of sweets). The index comprises 16 questions in the form of “yes or no”: questions denoting a negative connotation are assigned a value of -1 and those with a positive aspect $+1$. Total score ranges from -4 to 12 and it is divided into three levels indicating different levels of diet quality: (1) ≥ 8 , optimal Mediterranean diet adherence; (2) $4-7$, improvement is needed to adjust intake closer to the Mediterranean diet; (3) ≤ 3 , very low diet quality. It has been used so far in a variety of settings and countries [3-5].

2.2. Demographic characteristics of the participants

Between M23 and M34, 42 children participated in the study, with a median age of 12.6 (11.3, 14.4) years; 24 participants with diabetes, 4 with obesity and 14 participants without obesity and diabetes (control group). Regarding the anthropometric characteristics, the median Body Mass Index (BMI) was 21.1 (17.5, 26.2) kg/m^2 . Participants with obesity and diabetes had a median BMI of 31.1 (30.0,

41.1) kg/m² and 21.5 (18.5, 26.3) kg/m², respectively, whereas participants of the control group had BMI of 18.7 (15.7, 20.1) kg/m². (Table 1)

Table 1: Demographic and anthropometric characteristics of study participants

	All participants N=42	Control group N=14	Children with Diabetes N=24	Children with Obesity N=4
Age (years)	12.6 (11.3, 14.4)	12.9 (10.1, 13.7)	12.1 (11.0, 15.4)	15.6 (13.2, 17.3)
Sex (%female)	43.9	46.2	41.7	50.0
Weight (kg)	50.1 (36.0, 68.8)	45.7 (36.0, 53.2)	50.5 (32.2, 71.7)	75.6 (67.3, 112.8)
Height (m)	1.57 (1.4, 1.6)	1.58 (1.44, 1.61)	1.55 (1.44, 1.64)	1.6 (1.5, 1.6)
BMI (kg/m ²)	21.1 (17.5, 26.2)	18.7 (15.7, 20.1)	21.5 (18.5, 26.3)	31.1 (30.0, 41.1)

BMI: Body Mass Index

Continuous variables are presented as median (25th, 75th percentile) and categorical variables as relative (%) frequencies.

2.3. Dietary intake of the participants

In total, 37 participants completed the dietary evaluation. In specific, 37 completed an FFQ, 12 a 4-day food record and 34 the KIDMED.

2.3.1. Results from Food Frequency Questionnaires

37 children completed an FFQ; 10 children from the control group, 21 children with diabetes and 6 children with obesity. Median energy intake was 2,562 (2,003, 3,272) kcal/day, 44.6 (40.4, 48.6)% coming from carbohydrates, 15.7 (13.0, 19.3)% coming from proteins and 39.1 (34.6, 41.4)% from lipids. Children from the control group had a median energy intake of 2,464 (1,515, 3,770) kcal/day, children with diabetes 2,435 (1,965, 3,165) kcal/day and children with obesity consumed a median energy intake of 2,586 (2,250, 2,848) kcal/day (Table 2).

Table 2: Dietary intake as assessed with Food Frequency Questionnaires

	All participants N=37	Control group N=10	Children with Diabetes N=21	Children with Obesity N=6
Energy intake (kcal/day)	2,562 (2,003, 3,272)	2,464 (1,515, 3,770)	2,435 (1,965, 3,165)	2,586 (2,250, 2,848)
Macronutrient intake/day (%energy intake)				
Carbohydrates	44.6 (40.4, 48.6)	47.1 (36.8, 48.7)	43.9 (40.3, 46.6)	47.5 (44.4, 52.1)
Lipids	39.1 (34.6, 41.4)	40.1 (38.0, 43.0)	38.7 (34.8, 41.1)	33.9 (32.1, 37.2)
Proteins	15.7 (13.0, 19.3)	12.6 (10.5, 18.8)	16.7 (14.5, 19.5)	17.2 (15.2, 22.6)

Continuous variables are presented as median (25th, 75th percentile) and categorical variables as relative (%) frequencies.

2.3.2. Results from 4-day food records

In total, 12 participants completed the 4-day food records; 9 in-paper form and 3 in the app (data not shown). However, from the children completing the record in the app, only 1 is included in the analysis, as the remaining ones did not report adequate data for the analysis (see Discussion). Thus, in Table 3 dietary intake data are presented from 10 children (9 in-paper form and 1 in the app); 5 children with diabetes and 5 children from the control group, none with obesity.

Median energy intake was 1,745 (1,257, 2,056) kcal/day; children with diabetes had median energy intake 2,023 (1,745, 2,068) kcal/day and children from the control group 1,301 (920, 1,881) kcal/day. Regarding macro-nutrient intake, 38.1% (34.7, 48.7) of total energy intake was from carbohydrates, 43.1% (34.9, 46.0) from lipids and 18.3% (16.0, 21.6) from proteins. In Table 3 results on energy intake, macro and major micro-nutrient intake are presented.

Table 3: Dietary intake as assessed with 4-day food records

	All participants N=10	Control group N=5	Children with Diabetes N=5
Energy intake (kcal/day)	1,745 (1,257, 2,056)	1,301 (920, 1,881)	2,023 (1,745, 2,068)
Macronutrient intake/day (%energy intake)			
Carbohydrates	38.1 (34.7, 48.7)	48.3 (38.1, 51.8)	35.0 (32.5, 38.2)
Lipids	43.1 (34.9, 46.0)	35.2 (33.7, 40.0)	44.6 (43.4, 50.6)
Mono-unsaturated fatty acids	19.9 (15.5, 23.6)	16.0 (13.2, 18.5)	23.5 (21.1, 24.1)
Poly-unsaturated fatty acids	6.7 (6.0, 8.1)	6.6 (4.3, 10.0)	6.7 (6.5, 7.5)
Saturated fatty acids	11.2 (9.5, 14.3)	9.8 (8.5, 12.5)	13.1 (11.1, 16.7)
Proteins	18.3 (16.0, 21.6)	18.3 (12.9, 22.6)	18.2 (16.9, 21.6)
Micro-nutrient intake/day			
Vitamin C (mg)	37.2 (21.4, 60.6)	52.3 (17.4, 67.8)	23.8 (21.3, 57.0)
Calcium (mg)	590.2 (328.1, 708.9)	479.3 (258.2, 590.2)	691.2 (469.2, 845.2)
Iron (mg)	14.8 (7.8, 17.6)	8.0 (5.2, 12.9)	17.3 (15.3, 21.0)
Vitamin D (IU)	100.2 (57.2, 180.5)	66.3 (35.0, 100.2)	175.9 (110.3, 215.8)
Folate (µg)	282.3 (203.8, 419.8)	211.1 (105.3, 404.1)	293.9 (269.8, 446.1)

Continuous variables are presented as median (25th, 75th percentile) and categorical variables as relative (%) frequencies.

2.3.3. Results from KIDMED

Thirty-four participants completed the KIDMED questionnaire, with a median score of 4.0 (2.3, 6.0) units; 38% of participants had poor adherence to the Mediterranean diet, 44% moderate and 18% high adherence to the Mediterranean diet. (Table 4).

Table 4: Diet quality as assessed with KIDMED questionnaire

	All participants N=34	Control group N=7	Children with Diabetes N=21	Children with Obesity N=6
Total score (-4 to 12)	4.0 (2.3, 6.0)	6.0 (2.5, 6.0)	4.0 (2.0, 6.0)	8.0 (7.3, 8.0)
Poor adherence to the Mediterranean Diet (%)	38	43	43	17
Moderate adherence to the Mediterranean Diet (%)	44	57	48	17
High adherence to the Mediterranean Diet (%)	18	0	10	67

Continuous variables are presented as median (25th, 75th percentile) and categorical variables as relative (%) frequencies.

2.3.4. Stress assessment

Another assessment planned to be performed in Study I-Phase I is the validation of the Perceived Stress Scale for Children Questionnaire [6]. The validation procedure is the administration of the questionnaire to 50 participants of Study I twice, with a 15-day interval (the first time on site and the second one by telephone), and perform statistical analyses on the validity of the questionnaire. So far, the questionnaire has been administered to 48 children; 46 have received the stress assessment at time 0 and 44 at time 2 (after 2 weeks); 2 of the children refused to complete it again.

2.4. Discussion

In total, 42 participants were included in the first phase of Study I; 24 participants with diabetes, 4 with obesity and 14 participants in the control group. It should be noted that the initial aim was to recruit 120 participants (40 children in each group), but it was not reached, due to a delay in patient recruitment related to the COVID-19 pandemic, as the Pediatric Department of Ospedale San Raffaele hospital was closed from March to September 2020. However, the number of participants included allowed us to obtain a picture regarding the dietary habits of the participants and feed the machine learning analyses.

In total, 37 participants completed the dietary evaluation. In specific, 37 completed an FFQ, 12 a 4-day food record and 34 the KIDMED. Of the participants who completed the 4-day food record, 9 completed it in-paper form and 3 in the app. Although the initial thought was that all the participants would complete the records in digital form through the NUTRISHIELD app, this was not ready in the beginning of the study (the app was released in M32), so alternatively recording of dietary intake was done in paper form. Both methods of administration, i.e. the in-paper and the digital form, have pros and cons. The common difficulties in both methods are the burden of time to complete, and the

difficulties related to the estimation of portion sizes, the identification of food preparation methods, and the recall of foods consumed [7]. The most important advantage when incorporating technology into a dietary assessment method is that it is thought to improve dietary intake accuracy, be more appealing to younger populations, and reduce the burden of the reporter [8]. Also, the app allows individuals to record images before and after eating occasions, which helps to cross-check the foods consumed and the estimation of the portion size and, thus, the accuracy of the analysis.

As it may be expected, many participants did not comply with the instruction of the in-paper completion of the record (9 out of 42 completed the record), despite the efforts made by the study personnel with frequent reminders through phone calls. However, the completion of the records digitally through the app proved to be more burdensome for participants in this Study, as the data obtained were of low quality. In detail, participants who completed the record through paper, compared with the digital form, who completed more days (3.6 days in comparison with 3.0 days, respectively) and reported more meals per day (3.7 meals in comparison with 1.3 meals, respectively) (data not shown). What is more, although the instruction was that the meals reported in the app should be accompanied by photos of the meals, before and after consumption, only 1 child complied with this. Finally, none of the children completing the record in the app reported quantities, even though a separate field for the quantities was included. Thus, one may speculate that the digital completion of food record is difficult and not easily applicable for participants in the Study. On the contrary, 37 participants completed the FFQ, without reporting serious problems and burdens, thus we propose that in the second Phase of the Study, the Intervention Phase, the FFQ should be used. Of note, participants who completed the FFQ reported higher energy and macro-nutrients intake, compared with participants completed the food record; median energy intake from the FFQ was calculated as 2,562 (2,003, 3,272) kcal/day, whereas energy intake from the food records was calculated to 1,745 (1,257,

2,056) kcal/day. The discrepancy between the two methods may be attributed to the low quality of the data obtained from the food records, as opposed to the FFQ.

Another study conducted in Italy on children ages 8-9 years old found a mean energy intake of ~2,100 kcal/day and carbohydrate, lipids and protein intake to 45%, 40% and 14% of total energy intake, respectively (Martone, 2013). The discrepancy between the energy intake can be attributed to the younger children included in the study, i.e. 8-9 years old. Indeed, other studies in older Italian children have found higher energy intake, ~2,100-2,300 kcal/day (Leclercq, 2009).

Only 18% of the children reported high adherence to the Mediterranean diet, as assessed with the KIDMED questionnaire. Although other studies conducted in children have shown higher percentages of optimal adherence to the Mediterranean diet, up to 55% [9, 10], studies conducted in Italy have shown similar results with the ones observed in this Study, with optimal adherence ranging from 5.0 to 19.6% [11, 12].

2.5. Conclusion

The dietary assessment of the present study indicated that children's energy and macro-nutrient's intake are in accordance with the literature. When it comes to diet quality, these results indicate that adherence to the Mediterranean diet is moderate. Interesting findings emerged regarding the recording of food records, either in-paper or digital, with the traditional in-paper form performing better than the digital format. However, it should be noted that when comparing the in-paper food record with the FFQ, the latter seemed to perform better.

3. Dietary Assessment of lactating mothers in Study II- Baseline Phase

3.1. Short description of the study protocol

Study II is an observational, parallel group, non-randomized study in lactating mothers. It is being carried out at the University and Polytechnic Hospital La Fe (HULAFE) in Spain, between M21 and M43. The study protocol has been approved by the Ethics Committee for Biomedical Research of the Health Research Institute La Fe and written informed consent is obtained from all participants.

Study participants include the donors providing the Donor Human Milk (DHM) and three mother-infant groups. In specific:

- **Group A** consists of pre-term infants fed with Own Mother's Milk (OMM) (PT-OMM) and their mothers.
- **Group B** consists of pre-term infants fed with pasteurized Donor Human Milk (PT-DHM), in range of complete enteral nutrition (150 mL/kg/day), and their mothers.
- **Group C** consists of 25 full term infants receiving OMM (FT-OMM) and their mothers.

The study is divided into two phases:

- A baseline phase, referring to the period between the time of delivery and the time preterm infants receive Complete Enteral Nutrition (CEN) or the time full-term infants Recover of Birth Weight (RBW). During this period, all infants and their mothers are evaluated once. Regarding the dietary assessment, during this phase one 24-hour recall is performed for all mothers to assess energy intake as well as macro- and micro-nutrient intake and consumption of foods of specific food groups.

In detail regarding the 24-hour recall, trained researchers asked for all foods and beverages participants consumed the previous day, using the multiple-pass method [13]. Recall data were analyzed in terms of nutrients using the dietary analysis software Nutritionist Pro™ (2007, Axxya Systems, Texas, USA). Additionally, dietary intake was grouped into food groups, namely fruits, vegetables, bread/starch, meat/high fat, meat/medium fat, meat/low fat, meat/very low fat, milk/non-fat fat, milk/low fat, milk/full fat and other carbohydrate-rich foods.

- An observational phase, lasting six months and referring to the period that infants are fed with Human Milk (HM) and/or formulas or solid foods. During this period, assessments are arranged in M1, M2, M3, and M6 for all mother-infant dyad, in the hospital or the participants' home. Donors are assessed every time a bunch of milk is donated.

Regarding the dietary assessment methods during this phase, a 24-h dietary recall is performed in mothers by trained researchers, upon every visit, as described above. Moreover, a validated semi-quantitative FFQ is completed by mothers of PT-OMM, PT-DHM, and FT-OMM as well as by donors providing the DHM upon the donation of a milk bunch, on the first visit (M1) of the observational phase [1].

The FFQ is administered by trained personnel and it comprises 142 questions on the consumption of foods that are commonly eaten by the Spanish population throughout a year, including dairy products, cereals, fruits, vegetables, meat, fish, legumes, added fats, alcoholic beverages, stimulants and sweets. Using a 9-grade scale (“never or less than 1 time/month”, “1-3 times/month”, “1 time/week”, “3-4 times/week”, “5-6 times/week”, “1 time/day”, “2-3 times/day”, “4-5 times/day”, “≥6 times/day”) participants are required to indicate the absolute frequency of consuming a certain amount of food, expressed in g, milliliters or in other common measures, such

as slice, tablespoon or cup, depending on the food. The previous month is set as the timeframe. The FFQ is a questionnaire easy to use and is not expected to increase the burden of lactating mothers.

Based on the FFQ-responses, adherence to the Mediterranean Diet is evaluated by using the MedDietScore, a composite score calculated for each participant [14]. For food groups presumed to be part of the Mediterranean pattern (i.e. those with a recommended intake of 4 servings per week or more, such as non-refined cereals, fruits, vegetables, legumes, olive oil, fish, and potatoes) higher scores are assigned when the consumption is according to the rationale of the Mediterranean pattern, while lower scores are assigned when participants report no, rare, or moderate consumption. For the consumption of foods presumed to be eaten less frequently within the Mediterranean diet (i.e. consumption of meat and meat products, poultry, and full fat dairy products), scores are assigned on a reverse scale. As the sample of the study is lactating mothers, the original score was modified by removing the component regarding alcohol consumption. Thus, the range of this modified MedDietScore is between 0 and 50, with higher values of the score indicating greater adherence to the Mediterranean diet.

The current deliverable focuses on data collected during the Observational Phase and the first visit of the Baseline phase (M1), and for Donors upon the donation of the first milk bunch.

3.2. Demographic characteristics of the participants

Twenty-one individuals participated in the study, with a median age of 36 (35, 39) years; 12 mothers of PT-OMM, 1 mother of PT-DHM and 8 mothers of FT-OMM. 30% of participants had at least one chronic disease and 32% had a multiple gestation (Table 5).

Table 5: Demographic characteristics of participants

	All participants N=21	Mothers of PT- OMM N=12	Mothers of PT- DHM N=1	Mothers of FT- OMM N=8
Age (years)	36 (35, 39)	36 (35, 37)	40	37 (34, 41)
Chronic diseases (%yes)	30	22	0	44
Multiple gestations (%yes)	32	33	100	22
Weight (kg)	64.0 (58.4, 71.5)	64.0 (59.6, 70.0)	65	63.5 (54.5, 73.0)
Height (m)	1.61 (1.59, 1.65)	1.60 (1.57, 1.64)	1.50	1.63 (1.60, 1.65)
Receiving supplements (%yes)	60	88	0	25

Continuous variables are presented as median (25th, 75th percentile) and categorical variables as relative (%) frequencies.

3.3. Dietary intake of the participants

3.3.1. Results from 24-h recalls

At the Baseline Phase of Study II, 25 participants completed a 24-hour recall; 12 mothers of PT-OMM, 5 mothers of PT-DHM and 8 mothers of FT-OMM. Total energy intake was 2.252 (1.510, 2.472) kcal/day, with 40.0 (30.9, 42.4)% of energy intake coming from carbohydrates, 18.6 (15.4, 23.6)% from protein and 43.1 (38.0, 48.3) % of total energy intake coming from lipids. Regarding the consumption of foods of specific food groups, median fruit and vegetable consumption was 2.0 (0.5, 3.5) and 1.5 (0.5, 3.2) servings/day, respectively. (Table 6)

At the first visit of the Observational Phase, 28 participants completed a 24h-recall; 13 mothers of PT-OMM, 6 mothers of PT-DHM and 9 mothers of FT-OMM. Total energy intake was 2.171 (1.749, 2.391) kcal/day, with 38.5 (34.1, 46.6) % of energy intake coming from carbohydrates, 18.0 (16.6, 22.7) % from protein and 42.6 (36.0, 46.3) % of total energy intake coming from lipids. (Table 7)

Table 6: Dietary intake as assessed from 24-h recalls in the Baseline Phase

	All participants N=25	Mothers of PT- OMM N=12	Mothers of PT- DHM N=5	Mothers of FT- OMM N=8
Energy intake (kcal/day)	2,252 (1,510, 2,472)	1,990 (1,292, 2,452)	1,725 (1,364, 2,258)	2,436 (2,068, 3,091)
Macro-nutrient intake/day (%energy intake)				
Carbohydrates	40.0 (30.9, 42.4)	36.1 (28.5, 41.4)	41.7 (30.0, 42.3)	41.7 (37.5, 44.8)
Lipids	43.1 (38.0, 48.3)	42.1 (36.1, 49.5)	44.3 (39.8, 47.7)	42.7 (40.7, 47.1)
Mono-unsaturated fatty acids	18.5 (16.4, 22.8)	19.0 (16.7, 22.9)	21.5 (14.3, 23.7)	17.3 (16.4, 20.3)
Poly-unsaturated fatty acids	6.3 (5.7, 7.7)	6.6 (5.9, 8.6)	5.9 (5.2, 8.1)	6.4 (5.5, 7.9)
Saturated fatty acids	13.8 (12.7, 15.2)	13.6 (11.2, 15.0)	13.3 (12.7, 15.7)	13.9 (12.9, 15.3)
Proteins	18.6 (15.4, 23.6)	21.4 (16.2, 24.5)	20.1 (15.5, 24.4)	15.8 (14.3, 18.8)
Micro-nutrient intake/day				
Vitamin C (mg)	142.0 (68.1, 209.9)	153.5 (89.5, 237.0)	76.4 (34.8, 274.6)	102.2 (61.0, 159.8)
Calcium (mg)	716.9 (463.8, 880.7)	746.0 (477.9, 819.9)	392.0 (172.8, 697.8)	739.5 (554.3, 1,260.9)
Iron (mg)	13.6 (10.3, 18.9)	16.1 (9.0, 19.2)	11.4 (5.4, 14.0)	13.3 (11.2, 21.2)
Vitamin D (IU)	157.1 (75.6, 270.7)	130.1 (56.8, 222.7)	72.0 (2.7, 170.0)	316.0 (180.3, 373.7)
Vitamin E (mg)	2.3 (0.3, 8.8)	4.7 (1.5, 12.2)	2.2 (0.3, 6.3)	0.3 (0.2, 3.8)
Folate (µg)	360.4 (278.1, 411,1)	357.3 (300.8, 408.8)	340.9 (193.6, 434.0)	374.8 (27.6.2, 423.0)
Consumption of foods of specific food groups (servings/day)				
Fruits	2.0 (0.5, 3.5)	2.0 (0.1, 3.9)	2.0 (1.0, 2.7)	1.2 (0.5, 4.6)
Vegetables	1.5 (0.5, 3.2)	2.0 (1.0, 3.0)	2.5 (0.7, 5.5)	1.0 (0.0, 3.4)
Bread/starch	5.0 (3.2, 8.0)	4.7 (3.6, 7.2)	2.5 (1.5, 4.5)	8.7 (4.6, 9.4)
Meat/high fat	1.0 (0.0, 2.0)	0.5 (0.0, 1.9)	0.0 (0.0, 0.0)	1.5 (0.0, 3.1)
Meat/medium fat	4.0 (2.0, 7.5)	3.0 (2.0, 5.6)	7.0 (2.7, 11.7)	6.2 (1.6, 7.5)
Meat/low fat	0.5 (0.0, 2.2)	0.3 (0.0, 5.7)	0.0 (0.0, 0.0)	1.2 (0.0, 1.5)
Meat/very low fat	0.5 (0.0, 1.7)	0.7 (0.0, 2.0)	0.0 (0.0, 0.0)	0.7 (0.0, 1.0)
Milk/non-fat fat	0.5 (0.0, 1.0)	0.7 (0.1, 1.0)	0.0 (0.0, 0.2)	0.5 (0.0, 1.7)
Milk/low fat	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.5)	0.0 (0.0, 0.0)
Milk/full fat	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.7)	0.0 (0.0, 0.0)
Other carbohydrate-rich foods	3.0 (0.0, 6.5)	0.2 (0.0, 3.0)	4.0 (1.5, 6.2)	5.5 (2.2, 8.9)

Continuous variables are presented as median (25th, 75th percentile).

Table 7: Dietary characteristics as assessed from 24-h recalls in the first visit (M1) of the Observational Phase

	All participants N=28	Mothers of PT-OMM N=13	Mothers of PT-DHM N=6	Mothers of FT-OMM N=9
Energy intake (kcal/day)	2,171 (1,749, 2,391)	2,051 (1,554, 2,334)	1,947 (1,552, 2,300)	2,262 (2,068, 2,660)
Macro-nutrient intake/day (%energy intake)				
Carbohydrates	38.5 (34.1, 46.6)	39.2 (34.5, 44.6)	45.0 (34.0, 49.3)	36.3 (32.8, 49.2)
Lipids	42.6 (36.0, 46.3)	42.5 (32.7, 45.9)	40.7 (37.4, 42.8)	46.4 (39.3, 50.6)
Mono-unsaturated fatty acids	18.3 (16.0, 20.3)	18.6 (12.8, 20.0)	16.8 (16.1, 20.4)	19.2 (16.1, 21.0)
Poly-unsaturated fatty acids	6.4 (5.1, 8.3)	6.0 (5.0, 7.4)	7.0 (4.5, 11.9)	6.8 (6.2, 8.0)
Saturated fatty acids	11.9 (9.9, 15.2)	12.6 (9.7, 15.0)	11.6 (10.3, 11.9)	13.0 (10.1, 18.9)
Proteins	18.0 (16.6, 22.7)	20.5 (16.0, 23.2)	17.3 (14.6, 23.3)	18.1 (16.7, 20.1)
Micro-nutrient intake/day				
Vitamin C (mg)	108.0 (53.7, 241.5)	68.3 (28.1, 335.7)	248.2 (126.0, 614.3)	122.2 (82.3, 146.6)
Calcium (mg)	701.0 (488.6, 1,074.5)	588.4 (343.0, 903.0)	690.6 (440.4, 887.2)	1,036.1 (713.9, 1,682.4)
Iron (mg)	13.0 (11.2, 20.6)	12.8 (9.9, 21.8)	14.8 (8.5, 21.0)	18.1 (12.5, 22.3)
Vitamin D (IU)	187.9 (92.8, 264.6)	153.0 (73.6, 332.8)	180.7 (76.9, 255.6)	197.7 (158.3, 366.7)
Vitamin E (mg)	1.4 (0.0, 4.9)	3.7 (0.0, 6.7)	0.6 (0.1, 5.8)	1.4 (0.0, 5.3)
Folate (µg)	349.2 (246.0, 516.5)	310.2 (245.3, 448.1)	315.3 (184.0, 581.7)	438.0 (266.6, 584.7)
Consumption of foods of specific food groups (servings/day)				
Fruits	2.2 (1.1, 4.7)	2.0 (0.0, 4.0)	3.0 (2.5, 7.0)	2.5 (1.2, 5.0)
Vegetables	1.0 (0.0, 3.0)	1.0 (0.0, 3.0)	1.0 (0.0, 1.7)	2.0 (0.0, 3.0)
Bread/starch	6.5 (4.5, 8.9)	6.5 (4.5, 9.5)	4.7 (4.1, 6.7)	7.5 (4.7, 10.5)
Meat/high fat	0.7 (0.0, 1.9)	0.0 (0.0, 1.2)	0.0 (0.0, 0.0)	2.0 (1.0, 3.2)
Meat/medium fat	4.2 (1.6, 7.4)	4.5 (2.0, 7.0)	2.0 (0.0, 3.9)	6.5 (2.0, 9.0)
Meat/low fat	0.7 (0.0, 3.9)	0.0 (0.0, 5.2)	2.2 (0.4, 4.1)	0.0 (0.0, 2.0)
Meat/very low fat	0.0 (0.0, 1.7)	0.0 (0.0, 0.0)	1.0 (0.4, 4.7)	1.0 (0.0, 2.2)
Milk/non-fat fat	0.5 (0.0, 1.0)	0.5 (0.0, 1.0)	0.0 (0.0, 0.9)	0.0 (0.0, 1.2)
Milk/low fat	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.2)	0.0 (0.0, 1.0)
Milk/full fat	0.0 (0.0, 0.5)	0.0 (0.0, 0.7)	0.0 (0.0, 1.6)	0.0 (0.0, 0.2)
Other carbohydrate-rich foods	2.0 (0.5, 3.0)	1.0 (0.0, 3.0)	2.0 (0.4, 3.8)	2.5 (1.0, 3.7)

Continuous variables are presented as median (25th, 75th percentile).

3.3.2. Results from Food Frequency Questionnaires

In total, 32 participants completed an FFQ; 12 mothers of PT-OMM, 5 mothers of PT-DHM, 8 mothers of FT-OMM and 7 Donors. Median energy intake was 1.278 (929, 1.668) kcal/day, with 59.2 (54.2, 62.5)% coming from carbohydrates, 22.9 (19.3, 26.8)% from lipids and 19.8 (18.4, 21.6)% coming from proteins. Median MedDietScore was 30.2 (27.0, 33.0) units; mothers of PT-OMM had a median score of 28.0 (25.2, 32.7) units, mothers of PT-DHM 32.0 (30.5, 33.0) units, mothers of FT-OMM 31.9 (29.0, 33.7) units and donors a median score of 30.8 (26.0, 37.0) units. (Table 8)

Table 8: Dietary intake as assessed with Food Frequency Questionnaires

	All participants N=32	Mothers of PT- OMM N=12	Mothers of PT- DHM N=5	Mothers of FT- OMM N=8	Donors N=7
Energy intake (kcal/day)	1,278 (929, 1,668)	1,158 (873, 1,359)	1,413 (975, 2,005)	1,270 (1,022, 1,371)	1,397 (778, 1,907)
Nutrient intake/day (%energy intake)					
Carbohydrates	59.2 (54.2, 62.5)	56.8 (52.1, 60.5)	63.7 (56.3, 73.4)	61.5 (57.4, 67.6)	57.7 (53.3, 67.8)
Lipids	22.9 (19.3, 26.8)	24.2 (20.1, 28.0)	21.8 (18.1, 25.3)	21.1 (17.7, 24.8)	23.8 (17.8, 26.9)
Proteins	19.8 (18.4, 21.6)	20.1 (18.4, 21.3)	17.5 (13.2, 21.5)	19.6 (18.5, 21.5)	21.1 (18.8, 23.3)
Consumption of food groups (servings/day)					
Non-refined cereals	0.8 (0.1,01.4)	0.4 (0.0, 0.7)	0.7 (0.2,01.2)	1.6 (0.9, 2.4)	0.6 (0.0, 1.1)
Refined cereals	1.9 (1.2, 2.4)	2.1 (1.4, 2.7)	1.4 (1.0, 1.9)	1.9 (0.9, 3.2)	1.7 (1.4, 2.1)
Fruits	2.4 (1.0, 3.0)	1.6 (0.9, 2.3)	4.6 (1.0, 9.2)	2.3 (1.3, 3.9)	2.3 (1.0, 3.5)
Vegetables	2.8 (1.5, 3.8)	2.0 (1.1, 2.9)	3.9 (2.1, 6.0)	3.3 (1.8, 4.9)	3.1 (2.4, 3.9)
Legumes	0.5 (0.2, 0.4)	0.4 (0.2, 0.7)	0.3 (0.1, 0.6)	0.6 (0.2, 0.3)	0.4 (0.2, 0.3)
Fish and fisheries	0.5 (0.4, 0.7)	0.2 (0.1, 0.2)	0.2 (0.1, 0.3)	0.2 (0.2, 0.3)	0.2 (0.1, 0.3)
Red meat and products	1.1 (0.5, 1.7)	1.2 (0.3, 1.9)	0.7 (0.5, 1.1)	1.1 (0.5, 1.7)	1.3 (0.8, 1.6)
Poultry	0.2 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)
Full fat dairy products	1.8 (0.6, 2.8)	2.0 (0.7, 2.6)	2.3 (0.7, 2.6)	1.4 (0.2, 2.7)	1.5 (0.4, 2.5)
Low fat dairy products	1.7 (0.0, 3.1)	1.6 (0.0, 2.8)	0.9 (0.9, 1.6)	1.1 (0.0, 2.6)	3.0 (0.0, 4.9)
Adherence to the Mediterranean Diet					
MedDietScore (0-50)	30.2 (27.0, 33.0)	28.0 (25.2, 32.7)	32.0 (30.5, 33.0)	31.9 (29.0, 33.7)	30.8 (26.0, 37.0)

Continuous variables are presented as median (25th, 75th percentile) values.

3.4. Discussion

In total, 21 participants were included in the Observational Phase of Study II. Given the study is in progress, it is anticipated that more participants will be included.

Regarding the dietary assessment, in the Baseline Phase of Study II, 25 participants completed a 24-hour recall; 12 mothers of PT-OMM, 5 mothers of PT-DHM and 8 mothers of FT-OMM. During the first visit of the Observational Phase, 28 participants completed a 24h-recall; 13 mothers of PT-OMM, 6 mothers of PT-DHM and 9 mothers of FT-OMM. Furthermore, 32 participants completed an FFQ; 12 mothers of PT-OMM, 5 mothers of PT-DHM, 8 mothers of FT-OMM and 7 Donors. The discrepancy between the sample size of the Baseline Phase and the first visit of the Observational Phase is attributed to some participants not agreeing to participate immediately after giving birth, but at a later point; when the infants reached CEN or RBW for pre-term and full-term infants, respectively.

At baseline, according to the 24-hour recalls, women consumed approximately 2.200 kcal/day, with 40% of energy intake derived from carbohydrates, 43% from lipids and the rest from proteins (18%). When dietary intake was assessed via FFQs, the picture was not similar; women reported an energy intake of 1.300 kcal/day, with 59% of energy deriving from carbohydrates, 23% from lipids and the rest from proteins (20%). Previous studies assessing dietary intake through various means (24h recalls, food records, FFQs) suggest that the energy intake of nursing women ranges from 1.600 – 2.050 kcal/day [15-17]. In agreement with these studies, a recent systematic review of 32 papers on the dietary intake of lactating women in developed countries supports the energy intake ranges from 1.400 – 2.800 kcal/day, with a median energy intake of 2.100 kcal/day [18]. Disparities between the reported energy and nutrient intake when assessed by 24h recalls and FFQs have previously been reported in multiple studies. For instance, a Brazilian study supports poor correlation between FFQs and 24h

recalls for assessing diet quality [19]. Moreover, in a sample of 60 women, FFQs were found to provide lower values of energy and nutrient intake when compared to 24h recalls [20].

With regards to macronutrients, similar to the intakes of the sample herein, lactating women in developed countries have been known to consume a diet consisting 41 – 66% of carbohydrates, with a median intake of 50%, while median intakes for lipids and proteins are 35% and 16%, respectively [18]. These macronutrient intakes, along with the reported energy intake, suggest that women adhere to current dietary guidelines for the breastfeeding period, with most women consuming at least the average requirement for energy and macronutrients [18].

In terms of diet quality, the women of the present sample achieved a mean MedDietScore of 30/50, which implies moderate adherence to the Mediterranean dietary pattern. Previous studies on the subject are in agreement with our results, with most nursing women adhering to a diet of moderate quality [18, 21].

3.5. Conclusion

Overall, the women of the study herein consumed a diet mostly adequate in terms of energy and macronutrient intake. However, our results indicate room for improvement when dietary quality is concerned. The discrepancies in dietary data derived by 24h recalls and FFQs underline that assessing the dietary intake of this population group is largely dependent on the means of assessment.

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