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Executive Summary

The present deliverable entitled “NUTRISHIELD Public Final Activity report” is a publishable final activity report, covering main aspects of the work, objectives, results and conclusions, including the publishable results of the final plan for using and disseminating the knowledge.

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Definitions, Acronyms and Abbreviations

Acronym	Title
App	Application (software)
DoA	Description of Action
FFQ	Food Frequency Questionnaire
FTIR	Fourier transform infrared (spectroscopy)
HM	Human Milk
IR	Infrared
SWOT	Strengths, Weaknesses, Opportunities, Threats
T1D	Type 1 Diabetes
Tx.x	Task
QCL	Quantum cascade Lasers
WPx	Work Package

1. Introduction

1.1. Purpose and Scope

The NUTRISHIELD Final Activity report is a publishable final activity report, covering main aspects of the work, objectives, results and conclusions, including the publishable results of the final plan for using and disseminating the knowledge.

1.2. Document structure

The Sections of the deliverable are organised in the following manner.

- After the introductory **Section 1, Section 2** depicts an outline of the work NUTRISHIELD did analysers and the results the project had in this field.
- **Section 3** describes the necessity of the monitoring of biomarkers and identifies said biomarkers.
- **Section 4.** outlines some of the NUTRISHIELD ICT tools development processes and potential integration into nutrition schemes
- **Section 5** summarizes the results of the clinical trials and the methodology that was decided to be implemented on them.

1.3. NUTRISHIELD Objectives

NUTRISHIELD succeeded at creating a personalised platform for the young. The platform consists of novel methods & techniques, which analyse a wide range of biomarkers related to nutrition and health disorders. Based on findings, the platform then uses ICT, by expanding existing nutrition assistive mobile apps, in order to provide feedback and steering people towards a better nutrition. This takes into account the way each person responds to different nutrients and food types, by also analysing phenotype, genome expression, microbiome composition, health condition, mental & psychological condition, as well as financial capabilities for procuring food.

NUTRISHIELD supported the execution of three validation studies in clinical settings.

NUTRISHIELD aimed to deliver on the following objectives:

Deliver an innovative technology platform for personalised nutrition that people want to use.

The target was to create all the right component that led to a unified platform/framework for optimising personalised nutrition, which is user-friendly and engages the end user in a pleasant and non-intrusive/judgemental way.

Within the NUTRISHIELD app and dashboard are delivered. These dedicated to personalized nutrition tools have been designed in collaboration via the efficient collaboration between software developers, bioinformatics and AI experts, nutritionists and medical doctors. The NUTRISHIELD app and dashboard aims to fulfil the needs for the end users (patients) and doctors. The app offers an intuitive and efficient manner for patients to receive personalized nutrition advice, guidance on how to follow these advices (some of which are derived via complex machine learning algorithms), monitor the adherence to plan, as well as provide the health professional useful information on the nutritional habits of the patients, as well as the degree of adhesion to the personalized nutrition plan.

Develop a set of new tools for assessing biomarkers relevant to personalised nutrition.

The NUTRISHIELD consortium developed a urine analyser, a human milk analyser, and a breath analyser, aiming at bringing analytical capabilities to many practicing doctors, thus making personalised nutrition more widely adopted. The means to validate objective was the successful delivery of urine analyser, human milk analyser, and breath analyser.

Identify correlation between biomarkers and personalised nutrition

The aim here was to identify correlations between specific biomarkers and nutrition (e.g. Microtyping, metabolome, etc), while also deriving correlation between different biomarkers. This helped create an intricate map of factors that affect personalised nutrition, leading to a more powerful nutritional algorithm. The NUTRISHIELD consortium identified several important correlations focusing on microbiome characterization.

Validate the NUTRISHIELD approach in clinical controlled settings.

The target was to put the proposed approach to the test, to validate its scientific validity, and also test it in real-world conditions regarding its efficacy in proposing sustainable and healthy food choice.

The means to validate objective was the successful completion of the three (3) validation studies in clinical settings (at HULAFE, OSR and RU).

Ameliorate the health of people engaged in NUTRISHIELD personalised nutrition.

The overall end goal of the project is to lead to healthier and happier people, while proposing sustainable and pleasurable food choices.

NUTRISHIELD managed to establishment of a fact-based connection between dietary choices and health amelioration, via the development of new methodologies and the identification of new correlations between biomarkers and personalized nutrition, the development of new tools (analysers and other techniques) that enable the efficient measurement of important biomarkers, and the development of the NUTRISHIELD platform that enables the efficient use of the NUTRISHIELD algorithms by both the patients and the health professionals.

2. NUTRISHIELD analysers

2.1. Description of Work

It was the ambition of NUTRISHIELD to integrate several biomarker measurements to better understand the relationship between diet and health. This understanding was then integrated into a personalized nutrition algorithm. Several biomarkers needed therefore to be measured from several samples. Each of these analyses required its own protocol development, optimization and possibly calibration and validation.

NUTRISHIELD developed the protocols used for the analysis of metabolites in urine and human milk. Significant development was required to adapt chromatographic and mass spectrometry techniques to measure the panel of selected metabolites from two different matrixes. Vitamins were measured in human milk. To accurately measure a large panel of lipid and water-soluble vitamins from human milk, Ultra Performance Liquid Chromatography was used. Following optimization, they had to be validated using reference material. All main steps described in method optimization are now completed, and the NUTRISHIELD analytical partners have analysed the samples coming from the Clinical studies carried out as part of WP5, as planned within WP3. The distinct methodologies developed for the individual analysers are as follows.

2.1.1. Human Milk analysis

Human milk has received the benefits of millions of years evolutionary pressure to make it the ultimate nutritional matrix for the survival and development of newly born human babies. Its composition is highly complex and evolves as a function of multiple factors such as time from birth and dietary habits of the mother. While there is some knowledge available on breast milk vitamin content, the data remain surprisingly scarce and when considering the technical difficulties to quantitatively extract and analyse vitamin profiles.

The NUTRISHIELD project offers a unique opportunity to collect reliable data on vitamin content of breast milk and its evolutive profile over time in well characterized mother-baby pairs. State of the art analytical techniques coupled with expert vitamin extraction and sample preparation can provide

reliable datasets that can then be integrated with baby's growth. Another unique research opportunity lies in the unprecedented chance to integrate high quality quantitative datasets on human milk vitamin and faecal microbiome and metabolic profiles to raise new scientific hypothesis on possible dietary micronutrient modulation of the gut functional ecology in the human new-born.

The target analytes of human milk are total protein and the three most abundant proteins casein, lactoferrin and α-lactalbumin. The experimental setup used a commercially available EC-QCL and a path length of 26 µm. A balanced detection setup was applied to achieve a better S/N ratio and therefore a better accuracy and in the end a better limit of detection.

To quantify the target analytes a PLS model was set up and 10 wavenumbers were chosen with 13 calibration samples. Raw human milk needs sample preparation, since the fat globules in the milk can lead to light scattering and a shift of the peak position to longer wavelengths. This was achieved by using a commercially available sonicator.

2.1.2. Urine analysis

One of the goals of the NUTRISHIELD project is the development of novel monitoring devices using laser-based technology provided by project partner Alpes Lasers. With the help of The Medical Research Institute of Hospital La Fe and I.R.C.C.S. Ospedale San Raffaele, nutrition dependent parameters in urine have been determined. The requirements for the parameters chosen was the availability of distinct absorption bands in the mid-IR spectral region. Furthermore, the selection was motivated by unsuitable existing standard methods which were either time consuming or regarding high amounts of (hazardous) consumables.

After the analytes have been defined, the method for the quantification was developed by Technical University Vienna using an FTIR. Infrared spectroscopy is a non-invasive technology, where no alteration of the sample takes place, compared to alternative methods which use colour reactions followed by colorimetric measurements. The FTIR used for the method development uses a Globar as a light source. Changing the light source to a quantum cascade laser (QCL) has a lot of advantages: A QCL has a higher light density, which means that the path length of the flow cell can be increased and in correspondence with the Beer-Lambert law, the absorbance increases proportionally. A

thicker flow cell reduces the risk of clogging and results in a more robust device. With a higher light power, a lower limit of detection can be reached as well. All these advantages were used for the development of the urine analyser prototype developed by project partner Quantared.

In the NUTRISHIELD project, the analyser prototype was developed. It has an autosampler, where 4 ports can be used for the measurement of the samples. The other ports are connected to cleaning solutions, which ensure the proper usage and cleaning of the device, to reduce carry over. The needed sample volume is very low, 250 µL, and the device can measure two analytes simultaneously. The analyser prototype is portable with a weight of 13 kg and dimensions of 0.35 m × 0.355 m × 0.355 m.

Using mid-IR spectroscopy, the analytes measured was phosphate and creatinine. Measurements with a flowcell of 50 µm thickness and BaF_2 windows with a FTIR were conducted. Phosphate and creatinine in a buffer solution at different pH levels were measured, as well as different other urine components, like urea and sulphate. The optimum wavenumber for creatinine was 1350 cm^{-1} and for phosphate, whose peak position is dependent on the pH of the urine, either 940 cm^{-1} (pH = 5) or 1010 cm^{-1} (pH = 1). During these experiments, a white film on the BaF_2 windows was observed. After further investigation, the white film was identified as $BaSO_4$. This observation was important for the NUTRISHIELD analyser in choosing the suitable components. The options were:

- Using coated BaF_2 and measuring at 940 cm^{-1} at a pH of 5 (setting the pH at high accuracy is necessary)
- Using CaF_2 and measuring at 1010 cm^{-1} at a pH of 1 (no pH measurement necessary, since adding an excessive amount of HCl is ensuring the pH)

2.1.3. Electrochemical sensor by CSEM

The laser urine analyser needs a special treatment of the samples before being analysed which changes the samples pH. Furthermore, due to the small volume sample availability for infants, a miniaturized solution was necessary. For those reason CSEM was asked to develop a custom-made laboratory-based and portable system (sensor + reader) for detecting pH in urine. Such a device adds value to the project due to:

- Low-cost pH sensors
- Fast response time (3-5 minutes per sample)
- Small urine volume (50-100 µl) required for testing,
- The electrochemical readout system can be easily connected to a laptop and integrated to the IT NUTRISHIELD platform.
- The electrochemical detection technology can, if needed, be adapted for monitoring other clinically relevant biomarkers (e.g., glucose, uric acid, sodium, and potassium ions).
- Multiple urines analysis (6 urines per times)
- User-friendly Graphical user interface

State-of-the-art pH measurements with a pH meter needs a minimum of 2-5 mL sample. The amount of urine for the measurements is limited to 150 – 200 µl and therefore no conventional pH meter can be used in this case. CSEM is producing electrochemical sensors, which is a screen-printed electrode made up of 3 electrodes. To be able to measure the pH with the EC-sensor, as electrochemical Interface is necessary, as well as an open circuit potentiometry.

In this preliminary study, tailored disposable CSEM pH sensors have been prepared and tested for urine monitoring. The goal was to understand the sensor behaviour in terms of operational stability and establish the need of a sample pre-treatment or not after processing real urine samples.

The results obtained in this study demonstrated that the CSEM pH sensors are stable for approximately 1 hour of continuous measurements (i.e. calibration curves and urine monitoring) and they do not necessary require a pre-treatment (i.e. filtration). All the above-mentioned requirements totally cover the targeted disposable use of the pH sensors within the “NUTRISHIELD” project.

The pH sensor by CSEM was used as an additional tool for the NUTRISHIELD platform but will not be part of the integration into the urine analyser developed.

In the NUTRISHIELD project the synergies between the members is focused to give tailored nutrition advice to the population. In this way obesity and related diseases can be reduces and proper nutrition in the early stages of infant given.

Concerning the pH in urine, usually normal values go from 4.8 to 8.0 and unusual pH values is the first symptom of something not working properly in our body such as:

- Kidney failure
- Urinary tract infection
- Diabetic

Thus, within the NUTRISHIELD project, a rapid and accurate sensor able to be quantitatively measures pH in urine was developed, in parallel with its own reader, and made suitable with the NUTRISHIELD's Dashboard and Backend System

2.1.4. Breath Analyser

Using mid-IR spectroscopy, the concentration of hydrogen cyanide and methane is determined. The usable absorption lines were determined by modelling the infrared transmission properties of breath allowing the specification of the lasers to be provided by ALPES. The concentration of hydrogen was determined online using a commercial sensor. The measurement procedure for online and offline analysis of methane and hydrogen cyanide in exhaled breath is described.

Among the gases which can serve as biomarker for a specific health condition is hydrogen (H2). Hydrogen is indicative of certain conditions of gastro-intestinal disorders. Hydrogen and methane breath testing is a widely used diagnostic tool, based on the science that these gases are by-products of saccharide fermentation by gut microorganisms, rather than human metabolism. Glucose, lactose, and fructose are normally absorbed in the small intestine. Increased gas production following their ingestion is associated with malabsorption or premature fermentation due to excessive bacteria in the small intestine.

Hydrogen and methane are absorbed from the gastrointestinal tract, exhaled via the lungs and are thus measurable in breath. Increased gas production following ingestion of fructose or lactose is used to detect malabsorption of carbohydrates. Similarly, increases and ratio of methane and hydrogen in expired breath in response to ingestion of non-digestible lactulose, or glucose are used to predict small intestinal bacterial overgrowth (SIBO)*. So, the analysis of both, hydrogen as well as methane

in exhaled breath is required in order to assess the person's health condition (e.g. digestive issues related to SBIO, or malabsorption of lactose, fructose etc.).

The idea to use methane as a biomarker requires adding the capability to analyse Hydrogen to the NUTRISHIELD breath analyser.

Unfortunately, hydrogen can't be analysed by infrared laser absorption spectroscopy, the technology which is used for analysis for methane and hydrogen cyanide. Hydrogen is a homonuclear diatomic molecule which can't be detected by infrared radiation. Sensors for the detection of hydrogen are very common in industry; however, the concentration ranges of these sensors are in the low percentage because the usual purpose is the detection of an explosive mixture of hydrogen in air which is in the low percentage range.

The concentration of hydrogen in breath is 4 orders of magnitude less (in the ppm range), so more sensitive sensors are needed. These sensors need to be specific to hydrogen but should not respond to changes in temperature and moisture and should not respond to other gases which may be contained in exhaled breath. Raman spectroscopy, another type of molecular spectroscopy with high specificity could have been used. Unfortunately, the power of the laser required as well as the amount of hardware needed, and its associated cost excluded its use.

Finally, an electrochemical sensor similar to the type used in alcohol testing devices was selected. Though care has been taken to exclude effects caused by traces of alcohol or similar gases which could be present in breath, it is a good idea to follow a certain oral hygiene protocol when analysing exhaled breath.

2.2. Description of results

The following analysers were developed as a result of the project to accomplish the goals of NUTRISHIELD. Those prototypes will serve as a first step towards commercially viable products.

2.2.1. The NUTRISHIELD Breath Analyser

Figure 1 Breath Analyser Prototype

The user interface guides the user through a measurement. The analysis of breath consists of the following steps:

- Data entry
  - Enter patient-id as provided by NUTRISHIELD platform (to be performed by personnel)
- Analysis
  - Start the analysis by selecting “Analysis”.
  - The analysing system is checked, and internal measurements are performed (t< 1minute)
  - On command the person whose breath is to be analysed breathes into a mouthpiece in order to fill the cells with breath
  - A measurement of the breath in the cell is performed (t < 1 minute)
  - Data evaluation
- Calculation of concentrations
- Display analysis results
- Store or/and transfer analysis results with patient id

2.2.2. The NUTRISHIELD Urine Analyser

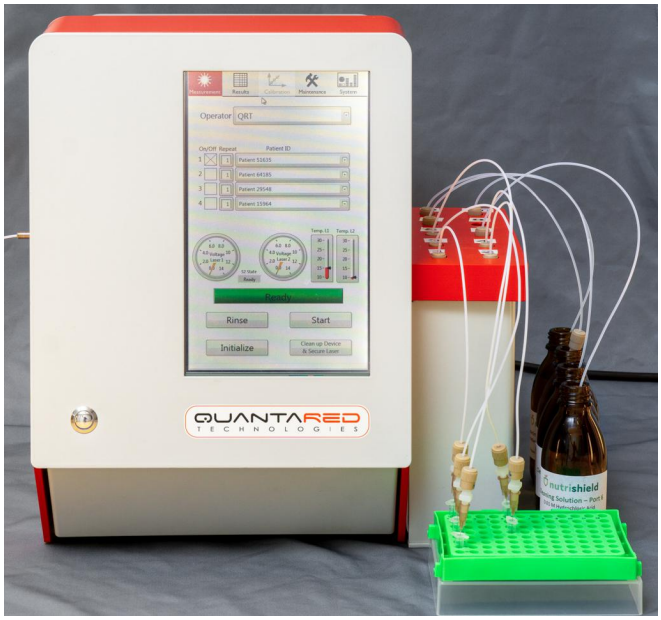


Figure 2 Urine Analyser Prototype

Urine analysis gives information about the medical state of the patient. With the quantified biomarkers the doctor can intervene if necessary to improve the patient's health.

Some standard methods for urine analysis are using special reagents to transform the analytes to a detectable modification. with which the analytes are reacting. Afterwards, a colorimetric measurement of the mixture is taking place. In contrast, the NUTRISHIELD Urine Analyser is not using any chemical reactions and therefore does not need any reagents. This facilitates a user friendly and safe operation. Apart from this advantage, the NUTRISHIELD analyser is measuring two health indicators at once. By taking natural internal standards into account, there is no need for a 24-hour collection of urine.

The NUTRISHIELD urine analyser prototype is aiming at the following points:

- Being portable
- Easy usability (touch display)
- Using a small sample volume
- No laboratory staff necessary
- Self-explanatory
- No reagents necessary
- No consumables

2.2.3. The NUTRISHIELD urine pH sensor

The user interface will guide the user through the measurements. Six urine can be measure simultaneously, 1 urine per instrument-channel. The analysis of urine consists of the following steps:

- Data entry
  - Enter for each patient the correspondent patient-id in the respective instrument-channels box (1-6)
- Analysis (A message is always displayed before each measurement (calibration 1, calibration 2 and urine sample) explaining which solution must be drop on the sensor)
  - Drop Cal 1 solution (80 ul) on the sensor (by covering all the 3 electrodes on it) and then start the calibration 1 by selecting "Start" – (measurement time 100 seconds)
  - Drop Cal 2 solution (80 ul) on the sensor (by covering all the 3 electrodes on it) and then start the calibration 1 by selecting "Start" – (measurement time 100 seconds)
  - Drop the urine sample (80 ul) on the sensor (by covering all the 3 electrodes on it) and then start the urine measurement by selecting "Start" (measurement time 100 seconds)
- Calculation of pH and display analysis results
  - Select "Processing" in order to process the data (<30 seconds)
  - A pH value is shown for each of the analysed urines
- Store or/and transfer analysis results with patient id

Figure 3 Urine pH Analyser Prototype

Device sensors
"ElchemMaster reader where 6 pH sensors are plugged in. After 2 points calibration procedure the urine can be analysed by covering the 3 electrodes of each sensor (working, reference and counter electrode) with the sample. 6 urines can be analysed at the same time".

2.2.4. The NUTRISHIELD Human Milk Analyser

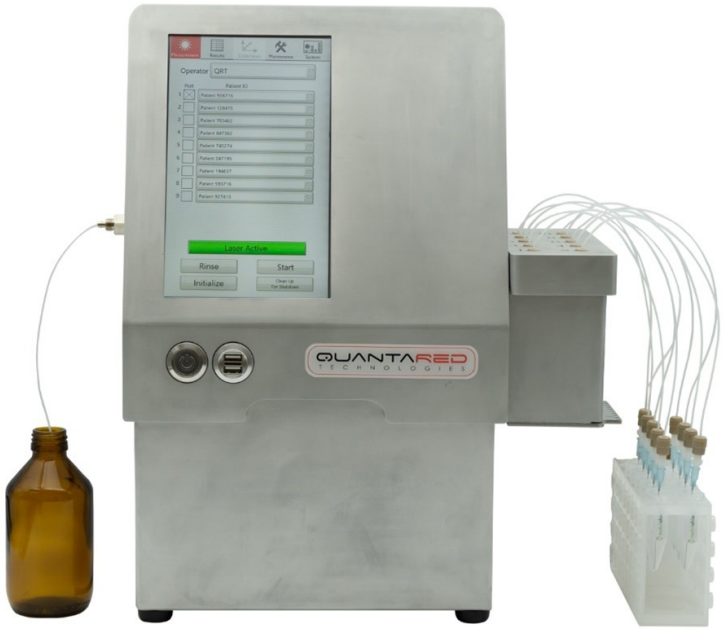


Figure 4 Human Milk Analyser Prototype

Human milk analysis gives information on its nutritional composition. It is very crucial for an infant's health, especially for pre-term infants, to feed them with high quality milk. The nutrition correlates with the development of the body and brain and can set its course of life.

During the research project QuantaRed Technologies GmbH is developing a completely new human milk analysis prototype with integrated novel laser sources. At the moment, the determination of the analytes requires two different methods, laboratory staff, time and consumables. The novel NUTRISHIELD human milk prototype will provide these determinations in one device.

The prototype aims at a completely automated procedure. The integrated autosampler provides the opportunity to measure 9 samples, without involvement of the end-user. During the measurement process, the device is cleaned properly with the cleaning solutions attached at the back. The prototype provides a lot of benefits for the potential users, amongst which are: Being portable, integrated touchscreen, no laboratory staff necessary and self-explanatory. Within the research project and the use cases the NUTRISHIELD Human Milk Prototype is further developed, tested and validated.

3. NUTRISHIELD biomarkers monitoring

3.1. Description of Biomarkers

3.1.1. Microbiome

Both obesity and Type 1 diabetes (T1D) are multifactorial chronic diseases characterized by inflammation at intestinal and systemic level. Studies in preclinical models and humans demonstrated that T1D and obesity are influenced by commensal gut microbiota composition. Moreover, recent evidence indicates that some dietary components are fundamental to regulate microbiota composition and promote a beneficial metabolic intestinal environment that reduces systemic inflammation.

Diet interacts with the human 'holobiont', i.e., commensal microbiota, in a person-specific way. From previous studies, several enterotypes have been strongly associated with long-term diets. In particular, the Prevotella enterotype has been associated with a high-carbohydrate diet, while the Bacteroides enterotype has been associated with a diet rich in animal proteins and fats.[1] A western diet high in fat, added sugars, choline, animal proteins or unhealthy plant-based foods (juices, refined grains, sauces, sweetened beverages)[2], has been shown to increase intestinal dysbiosis thus promoting inflammation. On the contrary, the Mediterranean Diet, high in fibber, vegetal protein, polyphenols, vitamins, minerals, anti-inflammatory omega-3 fatty acids, then vegetables, fruits, legumes, nuts, prebiotics, has been demonstrated to protect against inflammation through a restoration of the intestinal microbiota homeostasis.

This anti-inflammatory effect mostly occurs through the effect that specific foods and micro and macronutrients have on the production of metabolites by the gut microbiota. Food components get metabolized by beneficial gut commensal microbiota in the human gut to produce various types of

[1] Arumugam M, et al. Nature. 2011; 473:174. [PubMed: 21508958]
[2] Asnicar F, Berry SE, Valdes AM, Segata N. Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. Nat Med. 2021 Feb;27(2):321-332

microbial metabolites. Each microbial metabolite activates various pathways responsible for modulation of gut barrier and inflammation through the activation of membrane or nuclear receptors.[3] Dietary fibber, for example, represents a food component that is not digested at the level of the small intestine but passes directly through the ileum and colon, where it is partially or totally degraded by the microorganisms of the intestinal microbiota. As a product of degradation by microorganisms, monosaccharides are then fermented by the microbiota and converted into short-chain fatty acids or SCFAs, i.e. acetic, butyric and propionic acid. Subsequently, these metabolites are released and used by the host with important functions at the level of the organism. Specifically, SCFA induce differentiation of regulatory T cells with tolerogenic functions that reduce inflammation in the gut and systemically. thus, reducing SCFAs are mainly produced by Bifidobacteria, Lactobacilli, Roseburia, Eubacteria, Prevotella and Faecalibacterium. Other beneficial metabolites, such as indoles derivatives or microbial tryptophan metabolites which derive from the fermentation of green vegetables, has been shown to induce the AhR ligand activity that regulate a strong tolerogenic pathway thus reducing inflammation. On the other hand, high consumption of red and processed meat increases the risk of atherosclerosis and incurring cardiovascular diseases through an increase in the production of TMAO, a metabolite resulting from the transformation of carnitine by bacteria of the genus Bacteroides, Alistipes, Bilophila, Ruminococcus and Clostridia. More recently, it emerged that secondary bile acids, metabolites that are produced by commensal gut bacteria by modification of primary bile acids, also play important immune regulatory function and can increase differentiation of T regulatory cells and dampen inflammation.

Alterations of the gut microbiota composition have been found in individuals affected by T1D and obesity and, although a specific “pro-inflammatory” gut microbiota associated with those medical conditions have not been found, it is clear that modification of the gut metabolic environment through dietary intervention can favour immune tolerance and reduce inflammation in T1D and obesity. Although there is not yet a clear correlation between microbiota changes and dietary habits,

[3] Ghosh S, Whitley CS, Haribabu B, Jala VR. Regulation of Intestinal Barrier Function by Microbial Metabolites. Cell Mol Gastroenterol Hepatol. 2021;11(5):1463-1482.

recent studies have highlighted the capacity of specific food and macro/micronutrients to reduce inflammation by promoting a beneficial microbial metabolic profile.

The Nutrishield project focused at identifying dietary components and other lifestyle features (e.g., stress, physical activity) that can be associated to altered microbiota composition in diabetic and obese children. For this purpose, metabolomic and metagenomic analysis allowed us to link the presence and relative abundance of microbial species/microbial metabolites to intestinal inflammation, gut barrier damage and pathology (diabetes/obesity) in correlation with specific foods or nutrients. Based on a profile risk of each child related to dietary habits as well as microbiome and metabolome profiling, we were able to give a nutritional advice (Personalized Nutrition Algorithm) aimed at restoring a beneficial microbiome composition and reducing chronic inflammation associated with extra-intestinal diseases such as Type 1 Diabetes and Obesity.

3.1.2. Short-Chain Fatty Acids (SCFAs)

Short-Chain Fatty Acids result from bacterial fermentation of dietary fibres and resistant starch in our gut. They constitute the main source of nutrition for the cells in the colon and play an important role in our health. It was found that SCFAs can help reduce the risk of inflammatory diseases, digestive disorders, type 1 diabetes, and colon cancer as well as play an important role in the microbiota-gut-brain crosstalk.

Acetic acid, propionic acid, and butyric acid constitute the three most common SCFAs. Acetic acid, the shortest of the fatty acids, operates as an energy source for our muscles and also helps to keep the pH of the gut environment stable. Butyric acid has anti-inflammatory effects and contributes to cholesterol stabilization. Research has shown that it is involved in the treatment/prevention of Crohn's disease, colon cancer[4] and type 1 diabetes. Propionic acid, like butyrate, protects against

[4] Scheppach W, Bartram HP, Richter F. Role of short-chain fatty acids in the prevention of colorectal cancer. Eur J Cancer. 1995 Jul-Aug;31A(7-8):1077-80.

inflammations and may play a role in the prevention of colon cancer. Moreover, this SCFA is essential for our immune system and oral health.

Gut microbiota plays an important role in the bidirectional communication between the gut-brain axis, through various signalling mechanisms. Specifically, research has related the levels of SCFAs to mental and neurological issues[5].

SCFAs Food Sources

Dietary choices can affect the amount of SCFAs produced in our system. Higher quantities of SCFAs are present in high fibre and low-fat diets, compared to diets with a lower fibre intake[6] As SCFAs are generated through bacterial fermentation of dietary fibres, eating fibber-rich foods (fruits, vegetables, legumes, etc.) leads to increased production of these compounds. Moreover, foods made via bacterial fermentation like butter, cheese, and yogurt, also constitute rich sources of SCFAs[7].

It becomes clear that detecting the concentration of SCFAs in the human body can provide valuable information on one's nutritional status and therefore health, making timely and beneficial interventions easier.

In *Nutrishield,* case study III focused on the assignment of the (co)relation between various metabolites from exhaled breath and dietary quality for children with various diet habits and cognitive abilities. For this, RU had analysed the breath samples and together with CU performed multivariate modelling to relate these data to the nutritional status. Our preliminary results indicated the potential of breath metabolites and specifically of SCFA to predict nutritional status in children. The next step was to investigate how significant breath metabolites relate to specific elements of the diet (e.g. SCFA in relation to fibber intake).

[5] Silva, Y.P., Bernardi, A., Frozza, R.L. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Front. Endocrinol. 2020, 11, 25.
[6] Ou, J., Carbonero, F., Zoetendal, E. G., Delany, J. P., Wang, M., Newton, K., et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. Am. J. Clin. Nutr. , 2013, 98, 111–120.
[7] Shimizu H, Masujima Y, Ushiroda C, Mizushima R, Taira S, Ohue-Kitano R, Kimura I. Dietary short-chain fatty acid intake improves the hepatic metabolic condition via FFAR3. Sci Rep. 2019, 9(1):16574.

Furthermore, a non-invasive method via exhaled breath has been validated for measuring and analysing the SCFA levels[8]. This uses single exhalation and can be applied safely to all categories of participants, including children.

Table 1: SCFA, associated Health profits, and food source they can be found in.

SCFA	Health profits	Food Sources
Acetic acid	Energy source for muscles pH stabilization Appetite control	dairy products, bread, pasta liquid eggs
Butyric acid	Energy source for colon cells Anti-inflammatory effects Protection against cancer Neural system and brain protection	cheese, butter, milk
Propionic acid	Immune system protection Oral health Anti-inflammatory effects Protection against cancer	bread, pasta-noodles, aged cheese, eggs

[8] Henderson, B., Lopes Batista, G., Bertinetto, C. G., Meurs, J., Materić, D., Bongers, C. C. W. G., et al.. Exhaled Breath Reflects Prolonged

3.1.3. Vitamins

Nutritional patterns, the quantitative profile of nutrients and micronutrients that are actually consumed every day, play instrumental roles on the metabolic health trajectory from the first period of life to old age. Nutrients (carbohydrates, lipids, proteins, water) and micronutrients (vitamins, minerals and trace elements) interact at multiple levels of the biological organization from organs to cellular organelles orchestrating a complex network of biochemical processes and physiological functions. The term vitamin suggests the vital nature of micronutrient molecules belonging to this family. Classified according to their solubility, either in lipids (liposoluble), or water (hydro soluble), vitamins exert a myriad of functions such as the control of many enzymatic reactions via their cofactor roles, the regulation of nutrient absorption and of metabolic homeostasis, the modulation of gene expression, growth and development or the protection against molecular damages caused by oxidative stress. Vitamins are essential nutrients to humans, and several other species, due to either the absence of biosynthetic capabilities or the presence of some but at a limited rate that doesn't satisfy their metabolic requirements by the body. This is the reason why vitamins need to be absorbed from the diet to enable all the metabolic processes they are implicated in to be normally conducted. Vitamin intakes are thus crucial at every stage of life including in infancy.

Human milk has received the benefits of millions of years evolutionary pressure to make it the ultimate nutritional matrix for the survival and development of newly born human babies. Its composition is highly complex and evolves as a function of multiple factors such as time from birth and dietary habits of the mother. While there is some knowledge available on breast milk vitamin content, the data remain surprisingly scarce and when considering the technical difficulties to quantitatively extract and analyse vitamin profiles.

The NUTRISHIELD project offered a unique opportunity to collect reliable data on vitamin content of breast milk and its evolutive profile over time in well characterized mother-baby pairs. State of the art analytical techniques coupled with expert vitamin extraction and sample preparation provided reliable datasets that can be integrated with baby's growth. Another unique research opportunity was exploited in the unprecedented chance to integrate high quality quantitative datasets on human milk vitamin and faecal microbiome and metabolic profiles to raise new scientific hypothesis on possible dietary micronutrient modulation of the gut functional ecology in the human new-born.

4. NUTRISHIELD ICT platform

4.1. Description of Work

NUTRISHIELD aims at creating a personalised platform for the young. The platform will consist of novel methods & techniques, which analyse a wide range of biomarkers related to nutrition and health disorders. Based on findings, the platform then uses ICT, by expanding existing nutrition assistive mobile apps, in order to provide feedback and steering people towards a better nutrition. This takes into account the way each person responds to different nutrients and food types, by also analysing phenotype, genome expression, microbiome composition, health condition, mental & psychological condition, as well as financial capabilities for procuring food.

4.2. Description of results

The figure bellow presents the architecture of the NUTRISHIELD platform. The diagram represents the latest system architecture which is based on the users, components and applications identified in the course of the Project. At a high-level view, the NUTRISHIELD system consists of four major components, namely the Measuring Devices, the Dashboard (a web application), the Mobile Application and the Backend System.

The Backend System is a multi-functional component including many other sub-components such as databases, web services, the nutrition algorithm service and machine learning modules. It interacts with both the dashboard and the mobile application to provide the necessary support to their functionality. It should be mentioned that the Measuring Devices were used in clinical or lab settings and may not have direct connectivity to the rest of the system. These devices would therefore not be directly connected to the NUTRISHIELD platform. Respective measurements and analysis output originating from these devices is planned to be inserted to NUTRISHIELD by using the dedicated data entry forms which associate the input with the individuals it concerns.

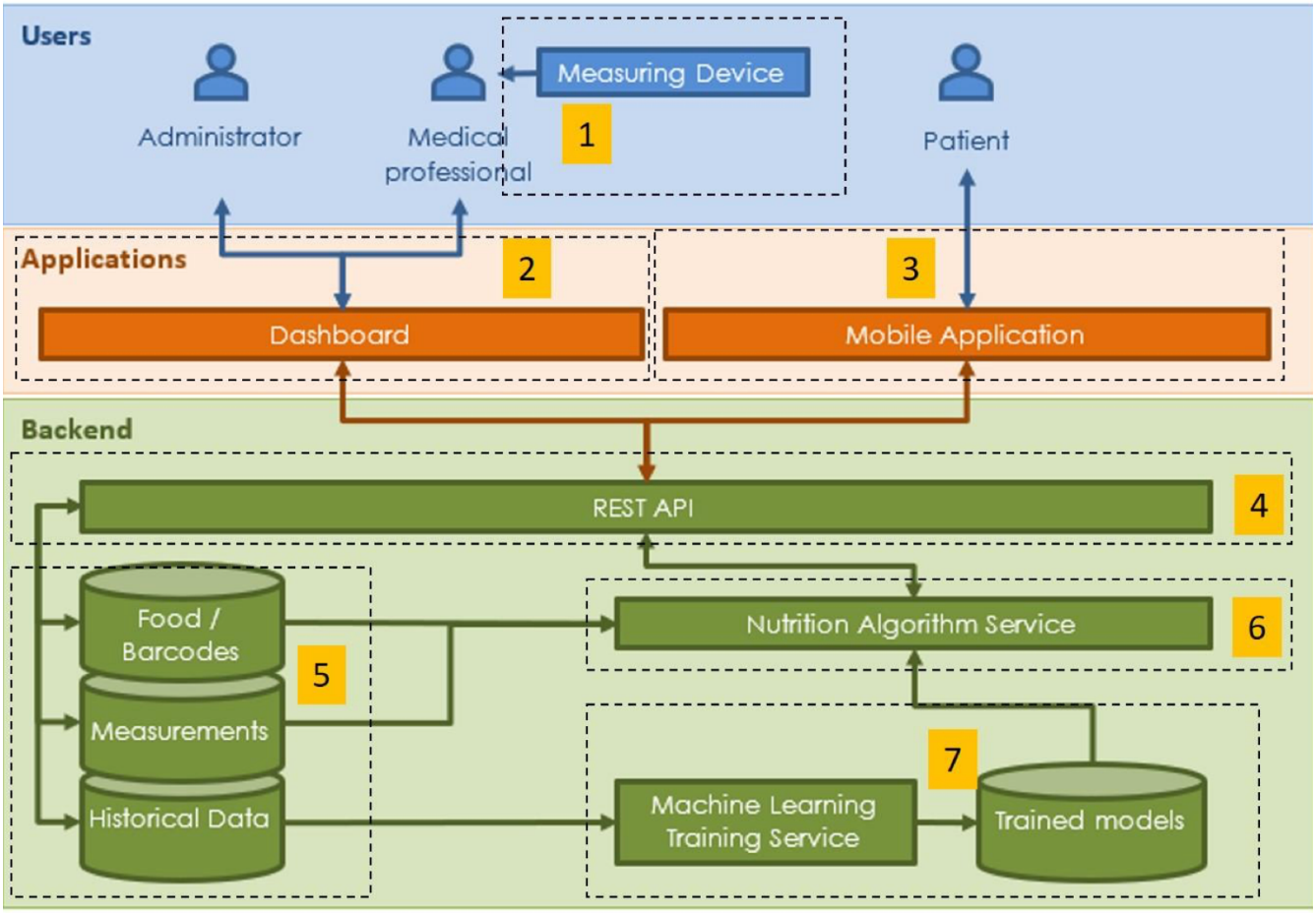


Figure 5 Architecture of the NUTRISHIELD platform

The **Measuring Devices (1)** consist of three prototypes for *urine*, *human milk and breath* analysis. The operator, measuring with the prototypes, will measure as instructed and will add the information from the prototypes to the NUTRISHIELD platform. Beside the concentration values for each

analyser, several additional info can be stored on the device, including measurement ID, Date & Time, Operator, Patient- ID/Sample ID, Absorbance and Status (Normal, Error, etc.).

The **Dashboard (2)** is a web application used primarily by medical personnel to monitor patients, upload measurements and prepare the dietary and activity plans. It interacts directly with the backend over the internet. Dashboard component will act as the main web application which is accessible by Administrators and Medical professionals. Dashboard component is the gateway to NUTRISHIELD Platform for Administrators (Data Managers and NUTRISHIELD Admins) and Medical professionals (Doctors & Lab Technicians). Each type of user is mapped into a distinct Role with certain level of functionality privileges and level of access on the NUTRISHIELD Data. Dashboard is available to registered users only as a Web Portal/App and will support functionalities summarized in the following list:

- **NUTRISHIELD Admins**: CRUD operations on Medical professionals' user accounts.
- **Data Managers**: CRUD operations on Food entries.
- **Medical professionals**: CRUD operations on Patient user accounts and Patients' measurements
- **Doctors**: CRUD operations on nutrition/activity suggestions, nutrition/activity plans and nutrition/activity diaries. Ability to send/schedule notifications towards patients' mobile app.

The NUTRISHIELD **Mobile Application (3)** is used by patients/users for receiving notifications regarding their dietary and activity plan and logging the food consumed towards keeping a food journal. Running on a smartphone which must be connected to the internet, it interacts directly with the backend, having a camera and enough storage to store meal consumption images and other information.

The **REST API (4)** represents a collection of RESTful API endpoints used by the dashboard and the mobile application. These endpoints can be used to transfer data and commands between the applications and various backend subcomponents. The logical layer of Rest APIs should be accommodated by a component that can provide web service features such as better governance on the system integration among NUTRISHIELD components and allow the integration of heterogeneous

systems that cannot be possibly integrated directly. VDP REST API is utilized as Middleware in order to allow integration of the following flows of Data:

- Mobile App to Databases (read/write operations)
- Dashboard to Nutrition Algorithm and vice-versa
- Dashboard to any external service (such as Push Notification Service)

For the data persistence needs of the NUTRISHIELD platform both relational SQL **Databases (5)** (e.g. PostgreSQL) and NoSQL (MongoDB) approaches were employed according to needs. Depending on the data and the related pre-defined or dynamic schemas where table-based or document-based approaches are necessary the appropriate database instance is used. Dashboard Application along with VDP Rest API require the presence of an SQL Database to hold the out of the box Database entities (Tables, Views, Procedures, etc.). The specific DB schemas had to be enhanced in order to hold customized entities where NUTRISHIELD data are stored (such as patients' measurements, nutrition personalized plans, etc.).

The **Nutrition Algorithm Service (6)** component represents the service that implements and makes available the NUTRISHIELD nutrition algorithm to the rest of the system. By leveraging relevant data maintained in databases and by receiving input and triggers from the Dashboard it produces personalised nutrition suggestions for patients. This component does not maintain any internal state but rather receives the necessary input to execute the algorithm and return the output. Communication with the databases to retrieve relevant data (measurements) coupled with other input provided by the Dashboard were used as parameters in the algorithm execution. A request for a nutrition suggestion is performed via a well-defined RESTful API that this component exposes. Endpoints of this API were used for both triggering the algorithm and providing the necessary input for its execution, as well as the retrieval of the resulting nutritional suggestion.

4.3. Description of the NUTRISHIELD app

To better describe the NUTRISHIELD app, we present the story of Anna, an imaginary girl of little age (about 10-year-old) using the NUTRISHIELD app. Here is her story:

Background story of Anna:

"Anna is a new user of the NUTRISHIELD app. She is about to use the app for the first time to receive nutrition recommendations. Anna visited the hospital where the doctors/health professionals collected all the info and data they need through food questionnaires, blood, urine, tool analysis, etc. The health professionals have processed the data locally at the hospital and have fully assessed her health condition and have prepared recommendations using the NUTRISHIELD algorithms. Now, both the doctors (they are health professionals in general, but Anna refers to them as doctors) and Anna need to find a way to make sure that Anna follows this plan. Anna should not be bored in the procedure, make sure she does not forget what she has eaten and also make sure she is honest in writing down what she ate."

4.3.1. Use of the model dietary plan

The output of the model dietary plan includes the number of food exchanges per food group that the child is advised to consume on a daily basis, to meet her/his needs, having taken into account basic demographic, anthropometric characteristics, and weight goal. This suggestion is accompanied by the list of exchanges and advise on the frequency of consumption and quality of food choices. The App visualizes this advice, having incorporated in the main menu a choice regarding Meals, where the recommended number of food exchanges is presented in the screen, in an interactive way. For each food group, a button shows the recommended number of food exchanges (1st Level of advice), and information about the portion size of one exchange within each food group (2nd Level of advice) is presented, along with advice about frequency/quality (3rd Level of advice). This functionality is presented below. Moreover, these buttons are movable, serving as a self-monitoring tool for the child: whenever (s)he eats a meal, the button is sliced down so many numbers as those that match the number of food exchanges consumed. Presentation of food exchanges

Figure 6, Figure 7, and Figure 8 comprise the visualization of the 1st 2nd and 3rd level of advice, namely the Model Dietary Plan incorporated into the mobile app, through an imaginary example.

"Let's assume that the algorithm suggested that Anna should consume within a day:

3 portions of Dairies

4 portions of Fruits

5 portions of vegetables

5 portions of cereals

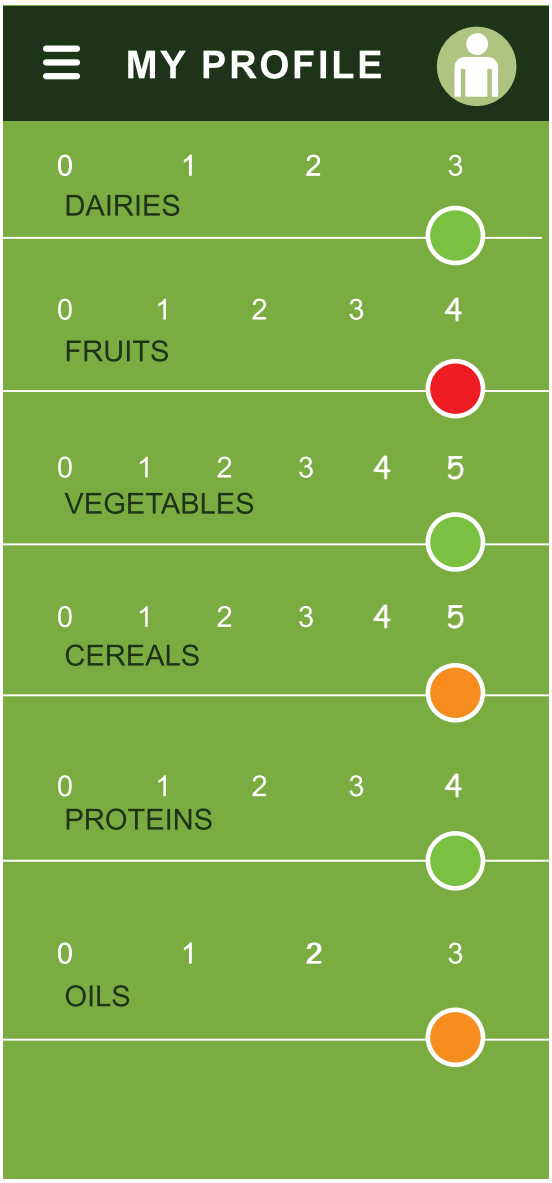
4 portions of proteins

3 portions of oils

Anna and her parents do not remember what these portions are, and they are confused because the doctors mentioned exchanges instead. They hope to understand better what Anna has to do using the Nutrishield app, which looks fun."

"Here are these portions/exchanges the doctor talk about. This is what she has to eat. However, as she visits the hospital yesterday, she and her mom do not recall what these portions are about. They only see that Anna should eat 3 portions of dairies, 4 portions of fruits, 5 portions of vegetables, etc. All circles are on the right with some number of tops indicating how much of each category she should eat."

Figure 6. Screenshot of the App which depicts incorporation of the 1st level advice of the model diet plan

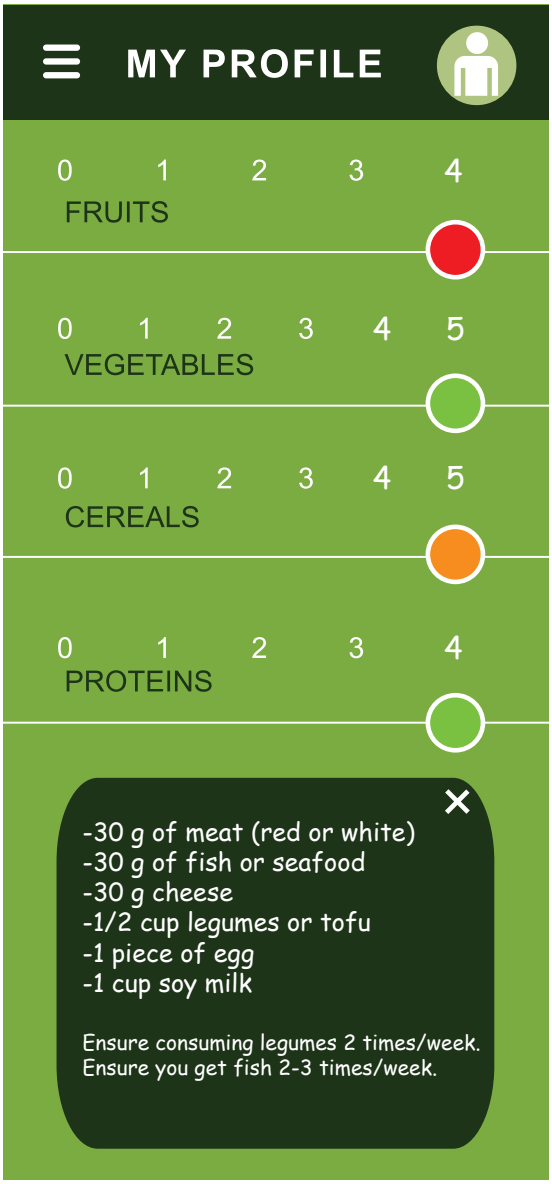


"Anna presses the circle just under the DAIRIES option and a balloon pops up. There it is! That's what the doctor has explained to her. This is what each of these portions are. There is a list of portions equivalent and underneath some more suggestions for her. She reads carefully what's there."

Figure 7. Screenshot of the App which depicts incorporation of the 2nd and 3rd level advice of the model diet plan for the Dairies food group

"Anna now tries the *proteins*. Meat, fish, but also legumes, all are here."

Figure 8. Screenshot of the App which depicts incorporation of the 2nd, 3rd and 4th level advice of the model diet plan for the Proteins food group

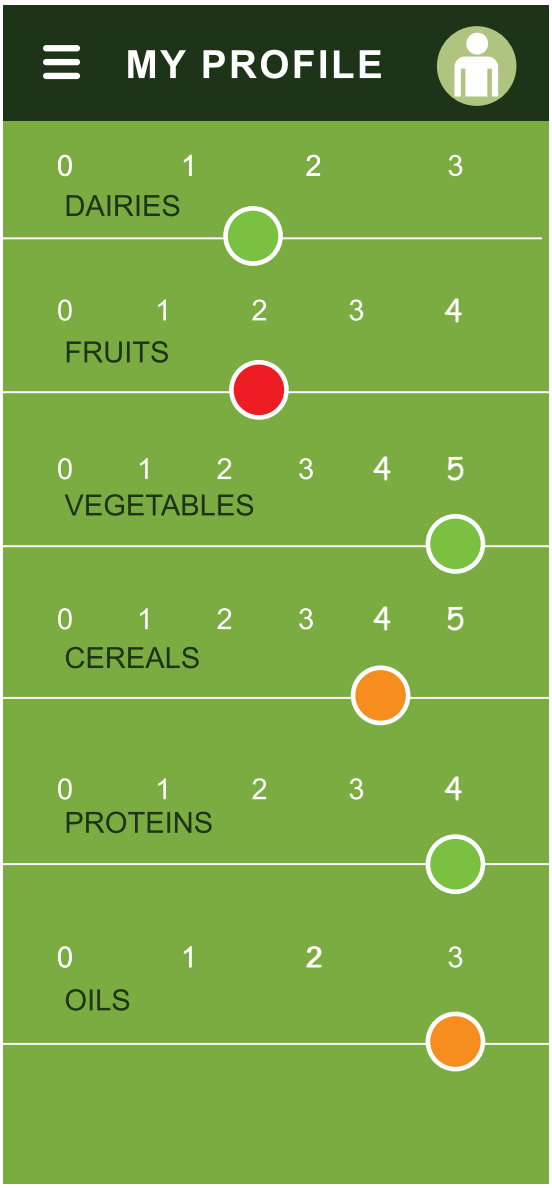


4.3.2. Self-monitoring of food exchanges

The child is asked to move the button indicator of each food group every time (s)he consumes a food item from that food group. In other words, is a count down, beginning from the numbers of food exchanges that have been recommended. Following are screen shots depicting this counting-down (Figure 9), which serves as a guide to organize future meals (Figure 10), ideally is nullified at the last meal (Figure 11), and it clears to restart the next day **(**Figure 12).

"It is time for Anna to have her breakfast. She is having some milk with corn flakes and some fruits. She now knows what the portions are so she and her mom can understand that she consumed some of her daily recommended portions. Anna moves the dairies slider by 1.5 to the left, the fruits slider by 2 to the left, and the cereals by 1. She didn't eat any vegetables, proteins, or oils, so she doesn't move these slides at all."

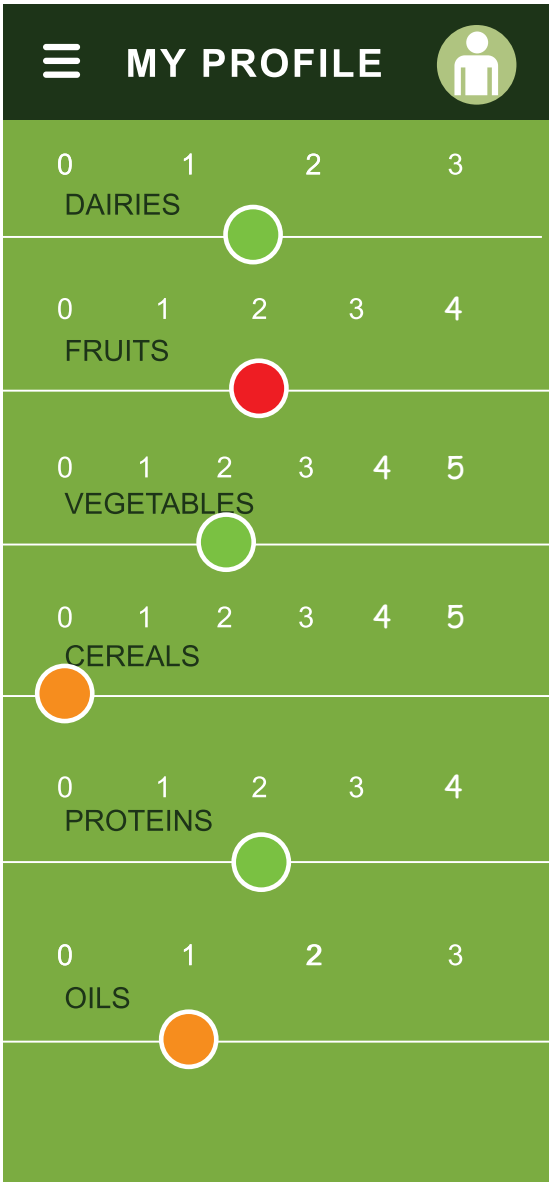
Figure 9. Screen shot of the App which depicts count-down of food exchanges for an imaginary breakfast.



Dissemination level: PU - Public

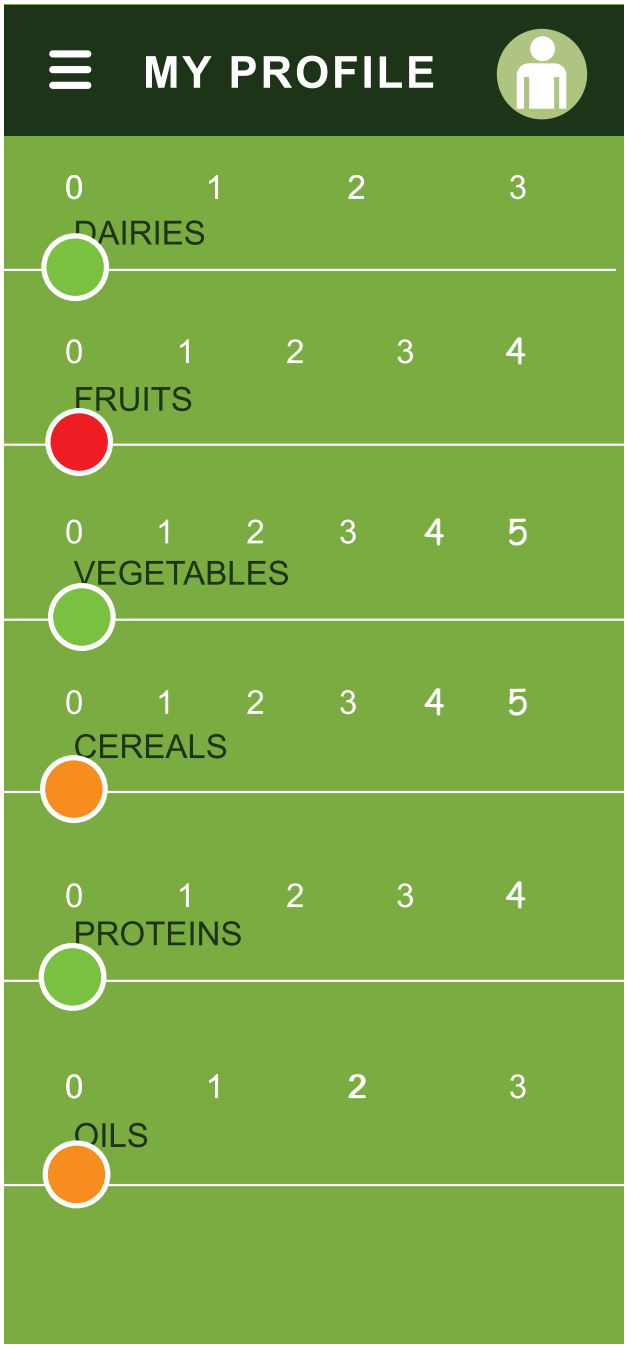
"For the rest of the day, Anna is not sure what she can eat further. She and her mom can have a look in the app to see what more she can eat."

Figure 10 Screen shot of the App which depicts the number of food exchanges left to be consumed at a random time during the day.



"By dinner, Anna has eaten all portions she was supposed to eat. The doctor will be very pleased. And if some day she ate a nice sweet and this nice extra portion of French fries, that's ok, she will take pictures of these meals as well so that her doctor can know."

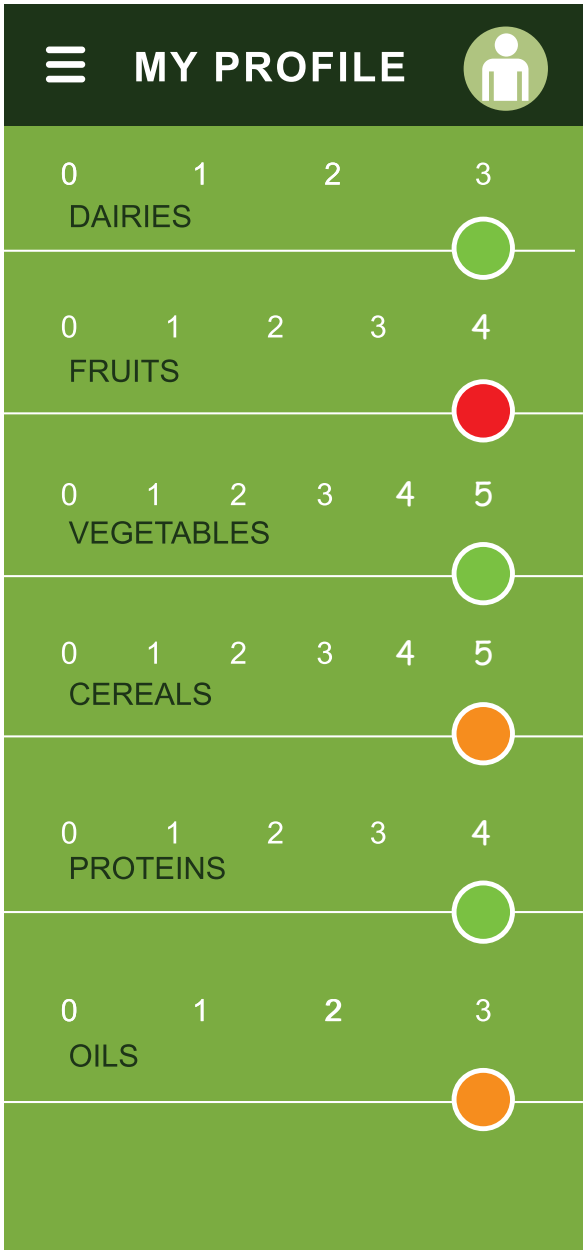
Figure 11 Screen shot of the App which depicts total consumption of the number of food exchanges recommended, meaning goal attainment



Dissemination level: PU - Public

"The next day, when Anna wakes up the morning, the app shows again the full portions she should eat today. There we go again!"

Figure 12. Screen shot of the App which depicts renewal of the number of food exchanges recommended

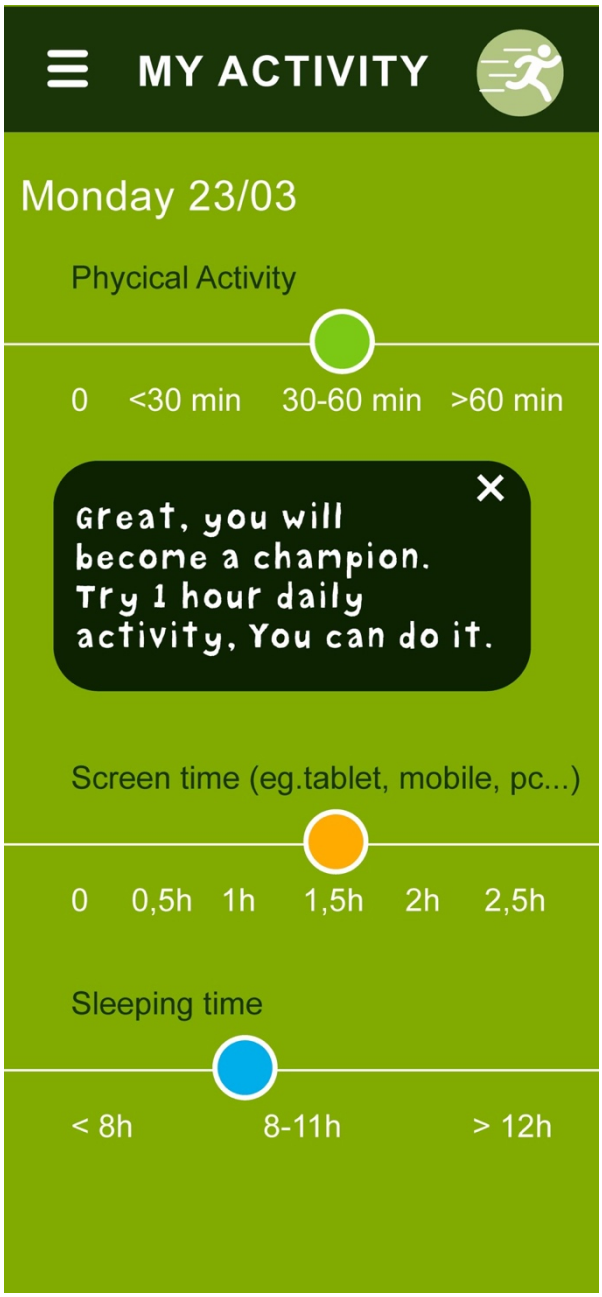


"Now her doctors know how well Anna follows their advice, and Anna knows what the doctors advised her to eat every day."

4.3.3. Physical activity monitoring

The App may also serve as a self-monitoring tool for physical activity, screen time and sleep **(Figure 13**).

Figure 13. Screen shot of the App which depicts self-recording of physical activity parameters.



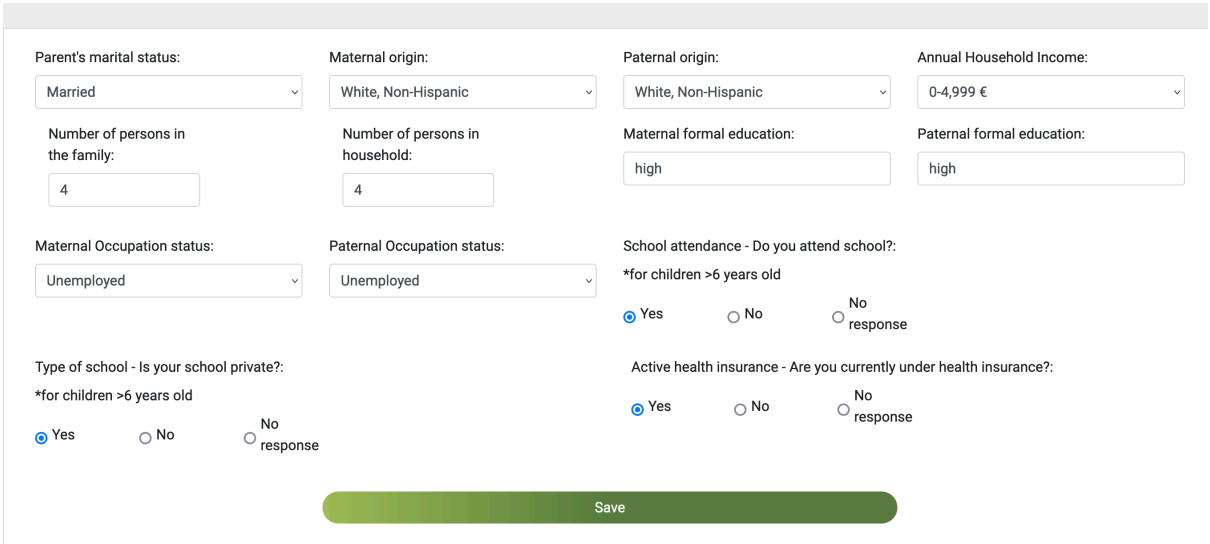
A video can be found in NUTRISHIELD's youtube channel:

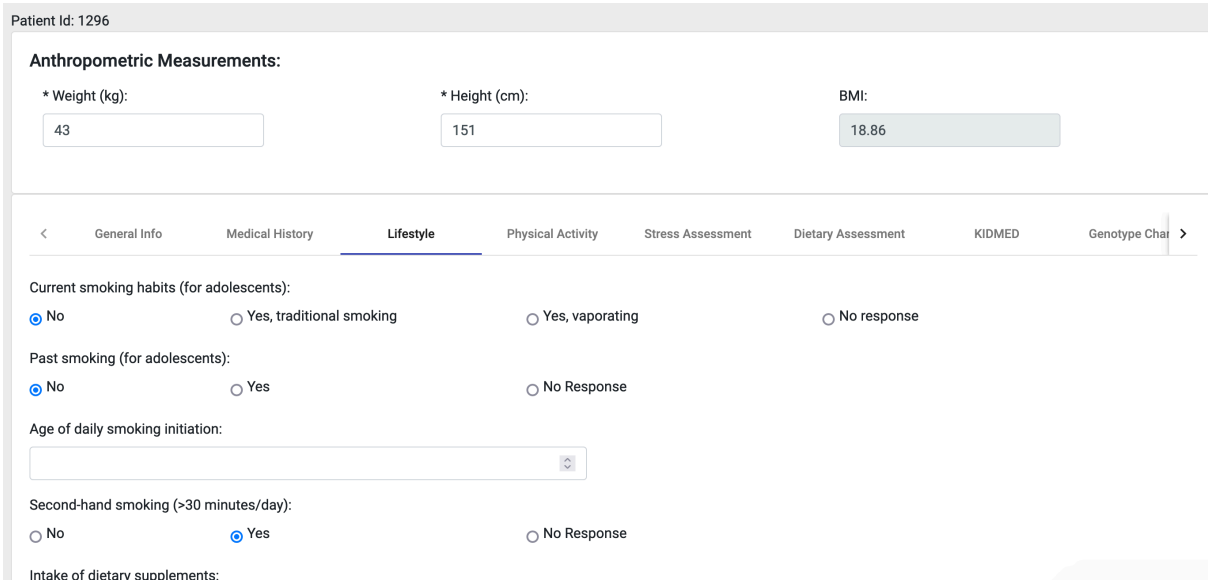
https://www.youtube.com/watch?v=76v9PZsV6xc&t=8s

4.4. The NUTRISHIELD dashboard

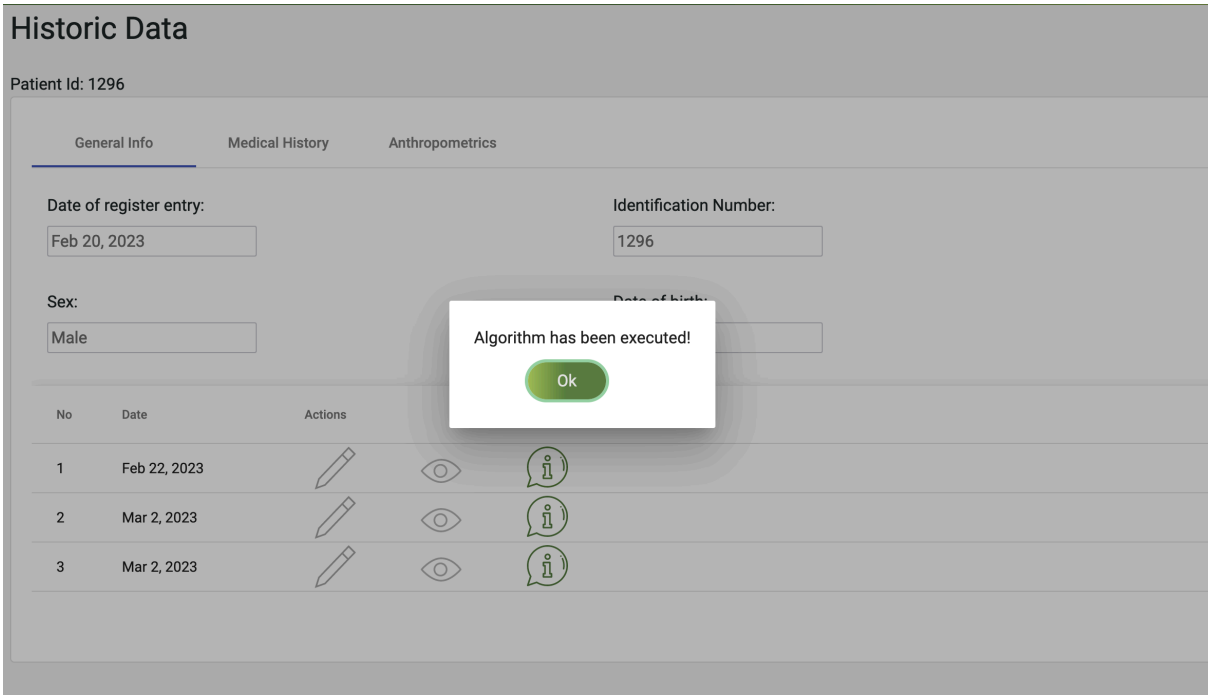
Next, the use of the NUTRISHIELD dashoboard from the view of Dr Alice.

Alice is a dietician working with children with diabetes which may or may not be obese as well. Anna is such a child and is treated by Alice.
Anna visited Alice at the hospital to be examined by a pediatrician at the clinic. Based on the results of Anna's exams, as well the answer Alice collected from her, Anna will provide several recommendations that aim to improve Anna's health and life by tailoring her diet in an optimal way.
During the first visit of Anna, Alice makes use of the NUTRISHIELD dashboard, a single-point access for all the information Alice needs for Anna. The dashboard is used to collect and store the medical info, process the data, and is also a convenient tool for Alice to send the dietary suggestions to little Anna, and of course her parents as well, through the NUTTRISHIELD app. All suggestions will be transferred directly from the dashboard to the smart phone of Anna's parents for easy access.
While at the hospital, Alice asks Anna a set of questions regarding several of her food habits and lifestyle choices. Of course, Alice is with her parents, they all provide all the answer Alice needs. Alice uses the dashboard to record all the answers to the questionnaires, along with the results of Anna's exams and other data she needs such as Anna's current weight and height. At the hospital, Anna also gives blood, urine and faeces samples to be analysed. All the results will be stored in the NUTRISHIELD dashboard for easy access and processing.

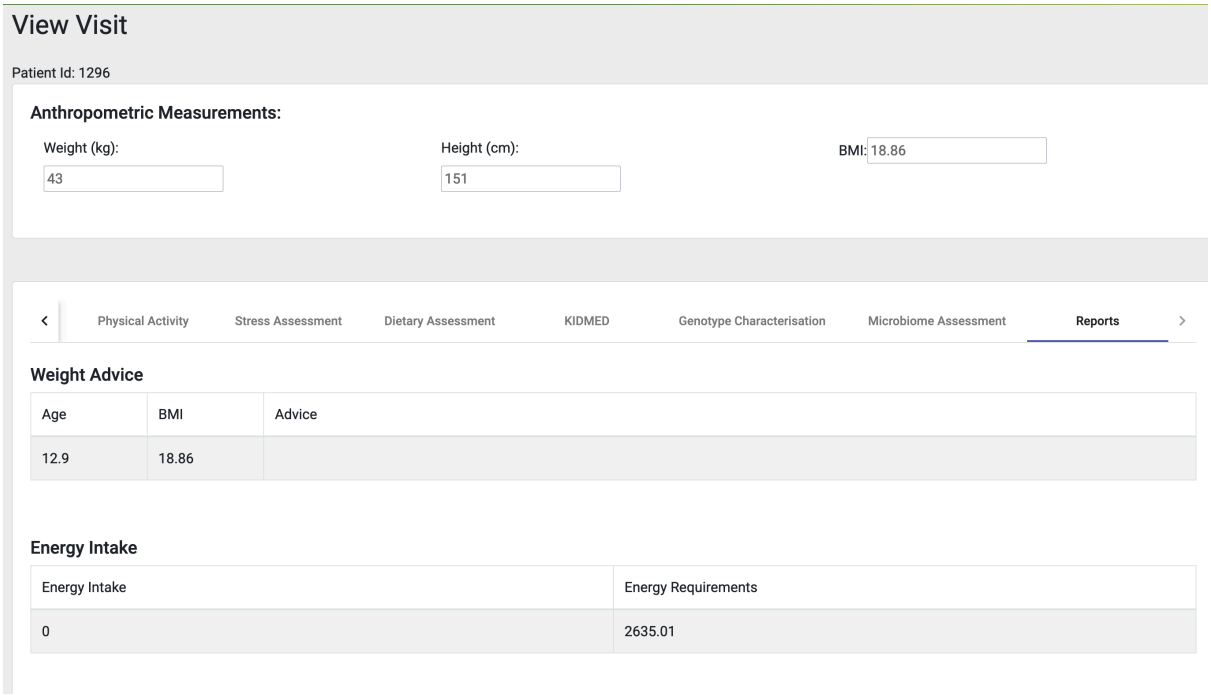




Once all results are available, Alice will process the data to provide the recommendations to Anna. By pressing the “i" button next to a visit in the historic data section, the dietary recommendation algorithm is executed.



The results can be found in the report section of each visit. This includes the number of exchanges, or portion, Annas should eat from each of the food group.



Next, Alice enters the Patient's activity section which allows her to both input the dietary recommendations to be send to Anna, and also monitors how well Anna follows the advices.

In the recommendations section Alice inputs the number of portions Anna is advice to consume every day. This is the outcome of the algorithm previously executed. Alice carefully sees the values of the algorithm and adapts if needed. But as explaining of these "portions" or exchanges is difficult, Alice adds an explanation for each of these.

Patient's Activity

Patient Id: 1296

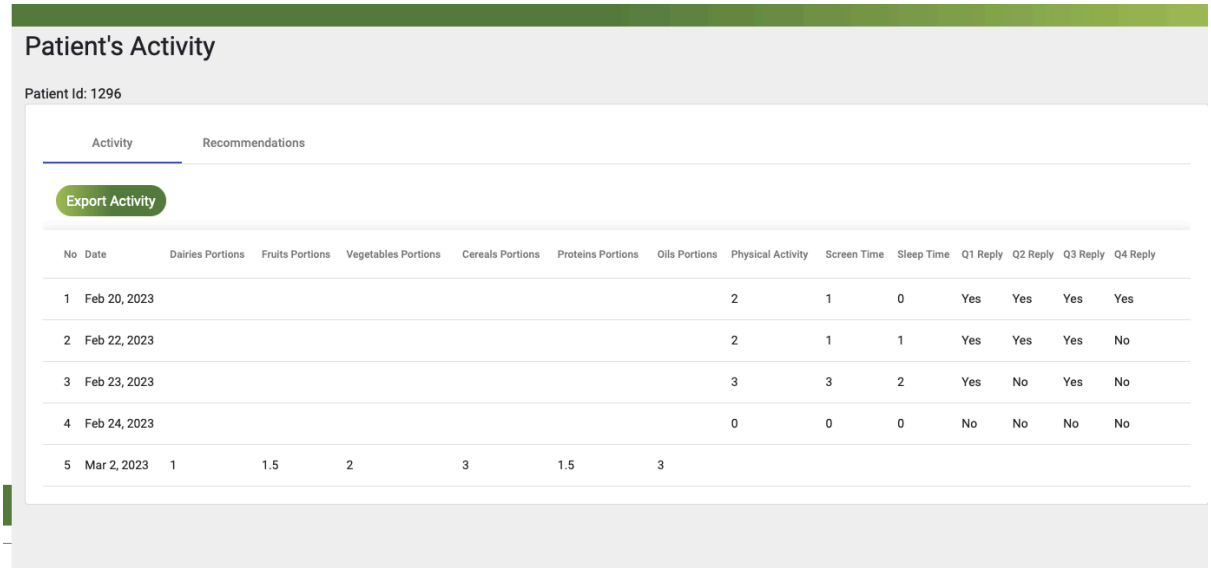
Activity | Recommendations

Add New Recommendation

No	Date	Dairies Recom Portions	Fruits Recom Portions	Vegetables Recom Portions	Cereals Recom Portions	Proteins Recom Portions	Oils Recom Portions	Dairies Recom	Fruits Recom	Vegetables Recom	Cereals Recom	Proteins Recom	Oils Recom	Question 1	Question 2	Question 3	Question 4	Actions
1	Feb 21, 2023	5	4	6	3	5	3	eat more yogurt this is correct line break here	eat apples	eat brocoλι	don't eat bread	eat meat	use olive oil	did you eat 2 apples today?	did you eat broccoli today?	did you eat 1 orange? line break testing	did you run 2 km today?	
2	Feb 22, 2023	5	4	6	3	5	3	eat more yogurt	eat apples	eat brocoλι	don't eat bread	eat meat	use olive oil	did you eat 2 apples today?	did you eat broccoli today?	did you eat 1 orange?	did you run 2 km today?	
3	Mar 1, 2023	5	4	3	3	4	5	fasdfgd dfads sfdsfa asdfd asdfsaf asf sfsad asdfa	asdfa	asdfa	asdfa	asdfa	asdfa	Did you eat an apple?	Did you have yogurt today?	Did you have yogurt today?	Did you have yogurt today?	
4	Mar 6, 2023	1.5	3	3	0	0	0	1 cup (=240ml) fresh milk 1.5-2% fat content, 1/2 cup condensed or evaporated low fat milk, 180-200 g yogurt from low fat milk	Apple (1 medium), Applesauce (unsweetened) (1/2 cup), Apricots (4 medium items), canned Apricots (1/2 cup), Banana (1 small), Blackberries (3/4 cup), Cantaloupe (1 cup), Cherries (12 large items), raw Figs (2 medium), Grapefruit (1/2 fruit), Grapes (17 small grapes), Kiwi (1 large), Mandarins (2 items), Mango (1/2 small mango), Nectarine (1 small), Orange (1 small), Peach (1 medium), Pear (1 medium), raw Pineapple(3/4 cup), canned Pineapple (1/2 cup), Plums (2 small), Raspberries (1 cup), raw Strawberries (1 ½	1 cup of fresh vegetables, or 1/2 cup of cooked vegetables (artichoke, asparagus, bean sprouts, beets, broccoli , Brussel sprouts, cabbage, carrots, cauliflower, celery, cucumber, eggplant, leek, mushrooms, okra, onions, peppers, radishes salad, wild greens, spinach, summer squash, tomato, tomato sauce from fresh tomato, tomato juice from fresh tomato,	1 slice of whole wheat or processed bread (30g), hot-dog buns (1/2 item), pita bread (1/2 item), flour (3 tablespoons), plain breakfast cereal (1/2 cup), corn flake breakfast cereal (2/3 cup), muesli (1/4 cup), rice (all categories) (1/3 cup), pasta (1/3 cup), potato, flesh only, baked or boiled (1 small item-85g), smashed potatoes, without fat (1/2 cup), boiled corn (1/2 cup), whole corn, (1/2 item -140g)	30 g of Beef [most beef categories (gound, meatloaf, corned beef, short ribs, prime grades etc)], Pork chops, top loin, boston butt, cutlets, Lamb (rib, roast, ground), Veal [Cutlet (unbreaded)], chicken (dark meat with skin), turkey (dark meat with skin), ground turkey or chicken, fried chicken (with skin), Fish: any fried fish, Sausage with <5g fat, Cheese: ricotta (60g), mozzarella, feta cheese, Eggs: 1 piece, 1 cup Soy milk	1 teaspoon of olive oil, Canola oil, peanut oil, Margarine, Mayonnaise, Corn oil, Sunflower oil, soybean oil, Butter, Avocado (1/8 of item), Olives (5 large or 10 small), almonds (10 items), cashews (6 items), peanuts (10 items), pecans (4 halves), sesame seeds (1 tablespoon), peanut butter (2 tablespoons), Bacon (1 slice)	Did you broccoli?	Did you eat yogurt?	Did you have apple?	Did you run for 30 minutes today?	

The same “recommendations” section is also used by Alice for another very important purpose. To provide Anna more specific, “personalized” advice. This is the outcome of the advanced machine learning algorithm that processes some important information from Anna. This includes the analysis of the microbiome and metabolomics, some very important factors that determines Anna’s treatment. All outcomes from the algorithms and other advanced tools are used by Alice to conclude

on which recommendations should be send to Anna. For example, Anna could be advised to consumed specific vegetables rich in some micronutrients she needs. Finally, in the recommendation section Alice can send specific questions to Anna to monitor her adhesion to the dietary plan. Through the app, Anna, with the help of her parents will be asked to reply to these questions every day. This way, Alice can monitor is Anna follows the specific recommendations and adjust if needed. Further to the replies to the specific questions, Alice can access a plethora of information on Anna's food intake and other factors through the dashboard. Using the NUTRISHIELD app, Anna reports what she ate each day, as well as her habits on using of screens, sleep time and exercise. All this information is available to Alice for processing, enabling her to both monitor how well Anna follows the advice, and change these advices if needed.



No	Date	Dairies Portions	Fruits Portions	Vegetables Portions	Cereals Portions	Proteins Portions	Oils Portions	Physical Activity	Screen Time	Sleep Time	Q1 Reply	Q2 Reply	Q3 Reply	Q4 Reply
1	Feb 20, 2023							2	1	0	Yes	Yes	Yes	Yes
2	Feb 22, 2023							2	1	1	Yes	Yes	Yes	No
3	Feb 23, 2023							3	3	2	Yes	No	Yes	No
4	Feb 24, 2023							0	0	0	No	No	No	No
5	Mar 2, 2023	1	1.5	2	3	1.5	3							

Dissemination level: PU - Public

Finally, during the next visit of Anna to the clinic, a new set of data, both medical and questionnaires data will be recorded by Alice and stored in the dashboard.

A relevant video on the story of Dr Alice can be found in the project youtube channel:

https://www.youtube.com/watch?v=U77ggFLPEf8&t=1s

5. NUTRISHIELD clinical studies

5.1. Description of Work

5.1.1. Clinical study I

Personalised Nutrition Of Young Individuals, With Obesity and/or Diabetes, aiming at ameliorating their health condition.

Objectives:

a) To evaluate the effectiveness of a “personalized” diet to young children with obesity or diabetes, versus the non-personalized approach.

b) Assessment of the effectiveness of an intensive Personalized Dietary intervention, encompassing genetic predisposition, biochemical markers and phenotypic information in a) children with obesity and type 1 diabetes (Intervention Group I) and b) obesity without type 1 diabetes (Intervention Group II), over the non-personalized approach (current lifestyle intervention) in children with obesity without type 1 diabetes (Control Group).

c) Assessment of the effectiveness of the suggested intervention in altering the concentration of novel biomarkers measured in breath, urine or faeces in the two intervention groups. Identification of possible links with obesity/ type 1 diabetes onset/or progression.

d) Assessment of the effectiveness of the suggested intervention in altering microbiome in the two Intervention groups vs. the Control group. Identification of possible links with obesity/ type 1 diabetes onset/or progression.

e) Cost-effectiveness analysis of the suggested dietary intervention for children with obesity or type 1 diabetes vs. other conventional approach. A short and concise description of the research methodology of the "NUTRISHIELD" clinical trial is presented, based on the CONsolidated Standards of Reporting Trials (CONSORT) Updated checklist (2017) for non-pharmacologic treatment interventions to refer to the most important methodological issues that should be addressed.

5.1.2. Clinical study II

Personalised Nutrition Of Lactating Mothers, Aiming At Augmenting The Nutritional Value Of Human Milk (HM). This Will Focus On Prematurely Born Infants, Which Have Increased Needs In Supplied Nutrients.

Objective: To assess the impact of the mother's diet on milk composition and growth and health status of preterm infants. Study design: The proposed study is a prospective observational cohort study (longitudinal, descriptive) to assess the relationship between maternal diet, nutrient levels in HM and genotype and microtype as well as metabolic response of the preterm infant.

5.1.3. Clinical study III

Personalised nutrition of young individuals, studying the link with development Cognitive development. This was linked to An ongoing trial performed by Donders institute.

Objective: To assess whether nutritional status can affect the outcome of spatial cognition training in the brain. Differences in brain activation patterns (fMRI) pre- and post-training was related to nutritional status, as measured by a composite score of the Dutch healthy diet index (i.e. a food frequency questionnaire assessing adherence to the Dutch healthy diet guidelines) and visceral adipose tissue quantified by abdominal MRI.

5.2. Description of results

5.2.1. Study I

5.2.1.1. 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 section of 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 crossmatch 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 ProTM 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, millilitres 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 index9; 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 were 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[101112].

5.2.1.2. 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,

[9] Serra-Majem, L., et al., Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. Public Health Nutr, 2004. 7(7): p. 931-5.
[10] Mariscal-Arcas, M., et al., Evaluation of the Mediterranean Diet Quality Index (KIDMED) in children and adolescents in Southern Spain. Public Health Nutr, 2009. 12(9): p. 1408-12.
[11] González-Valero, G., et al., Association between Motivational Climate, Adherence to Mediterranean Diet, and Levels of Physical Activity in Physical Education Students. Behavioral Sciences, 2019. 9(4): p. 37.
[12] Çağiran Yilmaz, F., D. Çağiran, and A.Ö. Özçelik, Adolescent Obesity and Its Association with Diet Quality and Cardiovascular Risk Factors. Ecology of Food and Nutrition, 2019: p. null- null.

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 2)

Table 2 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.

5.2.1.3. 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.

5.2.1.4. 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 3).

Table 3: 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.

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 were 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 4 results on energy intake, macro and major micro-nutrient intake were presented.

Table 4 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.

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 5).

Table 5: 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.

5.2.1.5. Stress assessment

Another assessment planned to be performed in Study I-Phase I is the validation of the Perceived Stress Scale for Children Questionnaire[13] . 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.

5.2.1.6. 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

[13] White, B., The Perceived Stress Scale for Children: A Pilot Study in a Sample of 153 Children. International Journal of Pediatrics and Child Health, 2014. 2: p. 45-52.

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[14] . 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[15] . 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

[14] Walker, J.L., S. Ardouin, and T. Burrows, The validity of dietary assessment methods to accurately measure energy intake in children and adolescents who are overweight or obese: a systematic review. Eur J Clin Nutr, 2018. 72(2): p. 185-197.

[15] Boushey, C.J., et al., Use of technology in children's dietary assessment. Eur J Clin Nutr, 2009. 63 Suppl 1: p. S50-7.

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].

5.2.1.7. Conclusion

The dietary assessment of the present study indicated that children's energy and macro-nutrient's intake were 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.

5.2.2. Study II

5.2.2.1. 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 was 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 analysed in terms of nutrients using the dietary analysis software Nutritionist ProTM (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[16].

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, millilitres 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[17] . 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

[16] Bountziouka, V., et al., Repeatability of food frequency assessment tools in relation to the number of items and response categories included. Food Nutr Bull, 2012. 33(4): p. 288-95.

[17] Panagiotakos, D.B., C. Pitsavos, and C. Stefanadis, Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis, 2006. 16(8): p. 559-68.

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.

5.2.2.2. 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 6).

Table 6 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.

5.2.2.3. Dietary intake of the participants

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 7)

Table 7 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).

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 8)

Table 8 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).

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 9)

Table 9 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.

5.2.2.4. Discussion

In total, 21 participants were included in the Observational Phase of Study II, given the unfortunate timing of the studies that was to be expected.

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[18][19][20] . 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. 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[21] . Moreover, in a sample of 60 women, FFQs were found to provide lower values of energy and nutrient intake when compared to 24h recalls[22].

[18] Ding, Y., et al., Dietary intake in lactating mothers in China 2018: report of a survey. Nutrition Journal, 2020. 19(1): p. 72.
[19] Aumeistere, L., et al., Impact of Maternal Diet on Human Milk Composition Among Lactating Women in Latvia. Medicina (Kaunas, Lithuania), 2019. 55(5): p. 173.
[20] Wang, D., et al., Analysis of dietary patterns and nutritional adequacy in lactating women: a multicentre European cohort (ATLAS study). Journal of Nutritional Science, 2021. 10: p. e17.
[21] Rodrigues, P.R.M., et al., Dietary quality varies according to data collection instrument: a comparison between a food frequency questionnaire and 24-hour recall. 2016. 32: p. e00047215.
[22] Olafsdottir, A.S., et al., Comparison of Women's Diet Assessed by FFQs and 24-Hour Recalls with and without Underreporters: Associations with Biomarkers. Annals of Nutrition andMetabolism, 2006. 50(5): p. 450-460.

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 of 41 – 66% of carbohydrates, with a median intake of 50%, while median intakes for lipids and proteins are 35% and 16%, respectively. 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[23] .

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[24].

[23] Di Maso, M., et al., Dietary Intake of Breastfeeding Mothers in Developed Countries: A Systematic Review and Results of the MEDIDIET Study. The Journal of Nutrition, 2021.
[24] Tabasso, C., et al., Adherence to the Mediterranean diet and body composition of breast-feeding mothers: the potential role of unsaturated fatty acids. Journal of Nutritional Science, 2021. 10: p. e63.

5.2.3. Study II

5.2.3.1. Background

A diet of sufficient quantity and quality is crucial for children's growth and mental development. An excess in energy intake, along with reduced energy expenditure, has contributed to the modern epidemic of pediatric obesity[25], while in the same time estimates of undernutrition remain concerning. This double burden of malnutrition poses great risk for children's current and future health and well being[26][27][28][29].

Beyond nutrient and energy intake per se, literature suggests that dietary quality has a major imprint on children's health as well. Lower dietary quality in children has been previously associated with worse sleep indices[30], higher prevalence of excess weight and central adiposity[31,32,33,34,35,36],

[25] Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet, 2017. 390(10113): p. 2627-2642.
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[28] Dewey, K.G. and K. Begum, Long-term consequences of stunting in early life. Maternal & Child Nutrition, 2011. 7(s3): p. 5-18.
[29] Park, M.H., et al., The impact of childhood obesity on morbidity and mortality in adulthood: a systematic review. Obes Rev, 2012. 13(11): p. 985-1000.
[30] Golley, R.K., et al., Sleep duration or bedtime? Exploring the association between sleep timing behaviour, diet and BMI in children and adolescents. Int J Obes (Lond), 2013. 37(4): p. 546-51.
[31] Grammatikopoulou, M.G., et al., Growth, the Mediterranean diet and the buying power of adolescents in Greece. J Pediatr Endocrinol Metab, 2018. 31(7): p. 773-780.
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[34] Archero, F., et al., Adherence to the Mediterranean Diet among School Children and Adolescents Living in Northern Italy and Unhealthy Food Behaviors Associated to Overweight. Nutrients, 2018. 10(9): p. 1322.
[35] Tognon, G., et al., Mediterranean diet, overweight and body composition in children from eight European countries: cross-sectional and prospective results from the IDEFICS study. Nutr Metab Cardiovasc Dis, 2014. 24(2): p. 205-13.
[36] Fernandez, C., et al., Association of Dietary Variety and Diversity With Body Mass Index in US Preschool Children. Pediatrics, 2016. 137(3): p. e20152307-e20152307.

breathing anomalies[37], and poor cardiometabolic health[383940]. Furthermore, evidence suggests that higher adherence to a variety of healthful dietary patterns is associated with enhanced neurocognitive development[41], reportedly more profound in boys [42], Last, better diet quality has been associated with improved academic performance[43].

Evidence is accumulating concerning the effects of dietary intake and quality of children in other health outcomes. For instance, the interrelationship between the diet and gut microbiota and its impact on children's health has been a potent area of interest in recent years. Diet has been described as a main driver of the composition and function of gut microbiota[44]; however, their relationship has been mostly studied in very specific pediatric populations (i.e. children with epilepsy, autism, gastrointestinal diseases)[454647]. Some results from healthy children have indicated that children with worse diet quality have reduced colonic short chain fatty acid fermentation compared to children with better diet quality[48]. Similar results have been produced in children adhering to a

[37] Garcia-Marcos, L., et al., Relationship of asthma and rhinoconjunctivitis with obesity, exercise and Mediterranean diet in Spanish schoolchildren. Thorax, 2007. 62(6): p. 503-8.
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[42] Haapala, E.A., et al., Associations of diet quality with cognition in children - the Physical Activity and Nutrition in Children Study. Br J Nutr, 2015. 114(7): p. 1080-7.
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[47] .Videhult, F.K. and C.E. West, Nutrition, gut microbiota and child health outcomes. Curr Opin Clin Nutr Metab Care, 2016. 19(3): p. 208-13.
[48] Kisuse, J., et al., Urban Diets Linked to Gut Microbiome and Metabolome Alterations in Children: A Comparative Cross-Sectional Study in Thailand. Frontiers in microbiology, 2018. 9: p. 1345-1345.

Western dietary pattern, compared to a traditional one[49][50]. Nevertheless, conclusions are yet to be drawn on how these findings affect pediatric development. In addition, cross-matching of dietary indices with other individual measures, such as breath markers, may also highlight potential targets for optimal growth.

5.2.3.2. Objective

The objective of this study was:

a) to evaluate the association of diet with health-related parameters, i.e. how the quality of the diet affects health related parameters (i.e. gut microbiota, breath analyses)

5.2.3.3. Methods

Overall study design, participants, measurements, sampling and statistical analysis are described in the following sections.

5.2.3.4. Study design

The proposed study was an observational trial, part of a trial in progress, performed by Stichting Kathoelike Universtiteit, in collaboration with Donders Institute, the Netherlands. It is entitled "Neural development of spatial cognition: an fMRI study on the effects of current and past nutritional status" (Principal Investigator: Dr. Esther Aarts, Donders Institute). The trial aimed at examining the relationship of nutritional status with the neural development of spatial cognition in 8-10 year old children. The part of the trial under NUTRISHIELD is observational, and takes place at its baseline. During recruitment (observational phase) participants were assessed once, as described below.

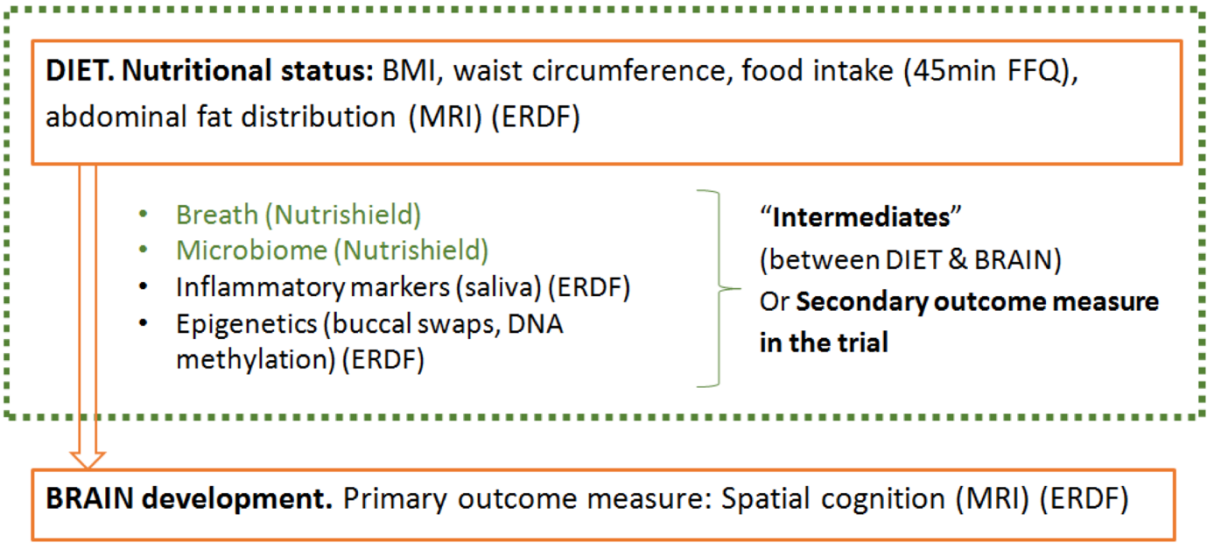
[49] De Filippo, C., et al., Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences of the United States of America, 2010. 107(33): p. 14691-14696.
[50] De Filippo, C., et al., Diet, Environments, and Gut Microbiota. A Preliminary Investigation in Children Living in Rural and Urban Burkina Faso and Italy. Frontiers in microbiology, 2017. 8: p. 1979-1979.

The trial is funded by the European Regional Development Fund (ERDF, PROJ-00405). Ethical approval for the ERDF trial has been granted by the Medical Ethical Committee of Radboud University Medical Center (date of approval: 22/05/2018; registration number: 2017-3923; NL-number: NL64464.091.17). Written informed consent had to be obtained prior to participation. All procedures regarding data security and handling in the context of NUTRISHIELD to be in absolute accordance with the principles set under WP 9, concerning Ethical Requirements, and more specifically stated on Tasks 7.1 and 7.2 and D 7.2.

5.2.3.5. Schematic study design

The design and the measures acquired at the baseline of the ERDF trial along with the role of NUTRISHIELD are presented schematically in Figure 9 below.

Figure 14 Role of NUTRISHIELD 1 within ERDE trial



The part of the joined action between NUTRISHIELD and ERDF highlighted in green.

5.2.3.6. Participants

The study sample was planned include 55 healthy children, aged 8-10 years old.

5.2.3.7. Baseline Study Phase

4.3.3.1. Measurements

Under the ERDF study protocol, all participants were to be be evaluated, including dietary intake, nutritional status, body composition, inflammatory markers and epigenetics, afterwards explored as intermediates in the relationship between the children's diet and spatial cognition. In collaboration with NUTRISHIELD, analyses of breath, and gut microbiome will be carried out in the study population.

Weight, height, computation of BMI, measures of abdominal fat by Magnetic Resonance Tomography (ERDF trial).

- Inflammatory assessment

Measured in children's saliva (ERDF trial).Inflammatory assessment is funded by an external company. Thus, using these measures under NUTRISHIELD warrants the company's approval.

- Genotype characterisation

Buccal swaps, DNA methylation patterns (ERDF trial). Epigenetics assessment is funded by an external company. Thus, using these measures under NUTRISHIELD warrants the company's approval.

- Microbiome assessment

Fecal samples from children were to be collected for studying the microbiome at baseline. The protocol followed is described in Deliverable 2.1.

- Breathanalyses

Breath measures of children were to be collected, and novel markers to be analyzed. The protocol followed is described in Deliverable 2.4.

5.2.3.8. *Sample size*

A sample size of 55 children, aged 8-10, has been found adequate to significantly detect expected outcomes of the ERDF trial.

- Sociodemographic information ERDF trial.

- Dietary assessment

45-minute Food Frequency Questionnaire (ERDF trial).

- Anthropometric assessment

Association of acquired measures with dietary measures will be explored. Additionally, the relationship in between acquired measures, other than dietary intake, was to be studied. Categorical values to be explored with Chi-square analysis. Student's t-test to be employed to test associations of continuous, independent variables. Correlations between continuous variables were to be tested with Spearman's r coefficient. A p-value of 0.05 is considered significant.

Statistical analyses were to be performed with the use of STATA statistical software (STATA Corp LLL, College Station, TX, USA).

Finally, additional statistical analysis with the view of deriving additional insights and influences in personalised nutrition will be presented in D2.5.

5.2.3.9. *Expectations and role to the project*

The collaboration of the ERDF study and NUTRISHIELD aimed to bring added value to both projects. This collaboration allowed for exploring associations of nutritional (abdominal fat measures) and other health-related parameters (epigenetics, inflammatory markers) not collected under NUTRISHIELD, with those studied under NUTRISHIELD. This joint approach was expected to greatly benefit the long-term vision of NUTRISHIELD, namely the evidence-based personalised nutrition for healthy youths.

Study III investigated the potential of using breath volatile as biomarkers to evaluate the diet quality and extrapolate on its impact on the cognitive function in children. To achieve this objective, breath samples from healthy children were collected and analyzed using advanced laboratory techniques (PTR-ToF-MS) and optical techniques and their correlation with various nutritional status indicators and a cognitive function test was investigated.

Multivariate modeling using breath volatiles and results from a food frequency questionnaire was performed to predict the body mass index (BMI) in children. A positive correlation between breath inflammation markers (acetaldehyde and ethylene) and the Flanker task error effect was observed, indicating the possibility for further investigation as biomarkers for cognitive function. Therefore, analyzing breath in children shows to be a potentially valuable tool to monitor 1) dietary habits and 2) the effect of diet, potentially also on cognitive development. These results are prepared for submission in the next months.

Furthermore, several experiments were conducted to validate the NUTRISHIELD-developed breath analyzer from Argos. The findings demonstrated excellent agreement between the different methods, thereby confirming the suitability the Argos breath analyzer to measure methane and hydrogen in breath of children.