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DELIVERABLE

D2.8 – Methodology and key metrics for validation & impact assessment

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Executive Summary

Clinical trials with a dietary component, besides an evidence-based, detailed approach to deliver the intervention to the study population, require an adequate methodology in order to reliably assess the outcome of the intervention. Relevant methods include thorough means and metrics, aiming to objectively quantify adherence to the intervention, as well as measure the impact of the intervention on the study population. The aim of this deliverable is to describe the methodology and key metrics that will be employed to validate adherence, and to assess the impact of the interventions that will be delivered through the NUTRISHIELD platform.

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Definitions, Acronyms and Abbreviations

Acronym	Title
FFQ	Food Frequency Questionnaire
HDL	High Density Lipoprotein
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IOTF	International Obesity Task Force
LDL	Low Density Lipoprotein
TMAO	Trimethylamine N-oxide
WP	Work Package

1. Preamble

When dietary interventions are concerned, thorough means aiming to objectively quantify adherence to dietary interventions, as well as measurements of the impact of the intervention on the study population are in order. The aim of this deliverable is to describe the methodology and key metrics that will be employed to validate dietary adherence, and to assess the impact of the interventions that will be delivered under NUTRISHIELD.

1.1. Objective of the deliverable

Deliverable D2.8 provides the full report on (i) the measurements that will be acquired (lifestyle, anthropometric, dietary and metabolic factors, changes in gut microbiome) to validate the impact on health of the studies performed under D2.7, (ii) the methods that will be employed to objectively measure dietary intake, and (iii) the means by which the satisfaction of the NUTRISHIELD platform and NUTRISHIELD mobile app will be assessed. Expected outcomes and the role of the present deliverable to the project are also discussed.

1.2. Content of the deliverable

Deliverable D2.8 includes:

- Procedures aiming to validate the impact on health status of the clinical studies presented in D2.7
- Objective measures of dietary intake, aiming to validate adherence to the dietary guidance
- Assessment of user-satisfaction

2. Validation of impact on health status

The NUTRISHIELD platform aims to create the conditions for reducing diet-related health disorders by fact-based personalized nutrition relying on measured biomarkers data and monitored food intake. The impact that the personalized nutrition has on health will be validated by measuring the biomarkers and the health-related factors, as described in Table 1.

2.1. Lifestyle factors

Levels of children's physical activity will be evaluated after the implementation of the platform-derived personalized diet, since it also includes promotion of physical activity levels. Increased levels of physical activity have been repeatedly connected in bibliography with improvements in health and cognition in children and adolescents [1-3].

2.2. Dietary factors

Another factor that affects children's health is the total energy intake and their diet's composition in macro-nutrients, especially of saturated and trans-fatty acid intake [4]. Selected micro-nutrients, such as vitamin D and iron have been associated with mental health of children [5,6]. What is more, increased consumption of fruits and vegetables [7-10], and dairy products[10-13] have been associated with favorable health outcomes. Finally, adherence to the Mediterranean Diet has been consistently related with health benefits in children [14].

2.3. Anthropometric and growth-related factors

Children's body weight, stature and BMI status will be compared to available growth charts, produced at the national or international level (i.e. WHO charts, extended IOTF cut-offs) [15-17].

2.4. Metabolic factors

For the possible improvement in the metabolic profile of children, blood glucose, insulin, A1C and HOMA-IR can be evaluated after the implementation of the platform derived diet [18,19].What is more, cardiometabolic risk factors such as total cholesterol, LDL cholesterol, HDL cholesterol, LDL/HDL ratio and triglyceride levels can be assessed for impact evaluation [20].

2.5. Gut microbiome

In the post intervention phase, changes in the gut microbiome are expected to occur [21]. Possible outcomes include a change in the quantity of colonic short fatty acid fermentation [22]. Other changes will be related to the overall composition of the microbiome. Indeed, a better suited diet is expected to increase the overall change in microbiome variety.

Table 1: Factors to be assessed for evaluation of impact on children's health

Factors		Expected outcome	Reference
Lifestyle factors	Physical activity	↑	[1-3]
Dietary factors	Energy intake	↓/↑	[15-17]
	Saturated and trans-fatty acid intake	↓	[4]
	vitamin D	↑	[5,6]
	Iron	↑	[5,6]
	Fruits	↑	[7-10]
	Vegetables	↑	[7-10]
	Dairy products	↑	[10-13]
	Mediterranean Diet	↑	[14]
Anthropometric and growth-related factors	Weight	↓/↑	[15-17]
	Height/length	↑	[15-17]
	BMI	↓	[15-17]
Metabolic factors	Blood glucose	↓	[18,19]
	Insulin	↓	[18,19]
	A1C	↓	[18,19]
	HOMA-IR	↓	[18,19]
	Total cholesterol	↓	[20]
	LDL cholesterol	↓	[20]
	HDL cholesterol	↑	[20]
	LDL/HDL cholesterol ratio	↓	[20]
	Triglycerides	↓	[20]

Factors		Expected outcome	Reference
Microbiome	A total of 336 different microbiome species, in detail described in WP3	↓/↑	[21]

3. Validation of dietary adherence through objective dietary intake measures

3.1. Dietary assessment

The dietary assessment of the participants is described in detail in deliverable D.2.7. In brief, participants in all 3 studies of D2.7 will complete at baseline, a semi-quantitative FFQ which assesses consumption of various food groups in the previous month. In addition, a 4-day diet record will be completed by participants of study I and study II of D2.7. Recall data will be analyzed in terms of nutrients using relevant dietary analysis software. Reported (subjective intake) dietary intake will be validated against proposed metabolites/ biomarkers that have been found to objectively reflect relevant food or food group consumption (objective intake). This process aims to validate reported dietary recall, and study I participant's adherence to NUTRISHIELD advice. A summary of proposed metabolites/ biomarkers to objectively quantify adherence to the advice of the NUTRISHIELD platform is stressed in Table 1.

Urine samples of the participants will be analyzed, according to standardized methods (for full details please refer to deliverables 2.2, 3.1). Consumption of dairy products (milk, lactose-containing dairy, cheese) will be validated by the presence of trimethyl-N-aminovalerate[23], galactitol [24], and glycines[25,26]in urine. Concentrations of isoflavones will be measured to validate soy consumption [27,28]. Intake of citrus fruit, other fruit and vegetables will be cross-matched with prolinebetaine[29] and flavonoids [30-32]. Metabolites of alkylresorcinol[33-35] will be used as biomarkers of whole grain cereal intake. 1- and 3- Methylhistidine, anserine and TMAO concentrations will be measured to validate

red meat, chicken, and fish consumption, respectively [36-39]. Concentrations of various acids, citrulline, taurine and isocitrate will be measured to assess non-alcoholic beverages consumption (tea, coffee, sugar-sweetened beverages)[40,41]. Last, resveratrol metabolites will reflect wine consumption [42,43].

Table 2. Urine biomarkers objectively reflecting specific food or food group consumption

Biological sample	Metabolite	Food or food group intake	Reference
Urine	Galactitol	Lactose containing dairy products	[24]
	trimethyl-N-aminovalerate	Milk & dairy products	[23]
	Isovalerylglycine Tiglylglycine Isobutyrylglycine	Cheese	[25,26]
	Isoflavones (and/ or genistein and daidzein concentrations)	Soy	[27,28]
	Prolinebetaine	Citrus fruits	[29]
	Flavonoids	Fruits & vegetables	[30-32]
	Alkylresorcinol metabolites	Whole grain	[33-35]
	1-Methylhistidine 3-Methylhistidine Anserine	Red meat	[36-38]
	Anserine	Chicken	[37]

Biological sample	Metabolite	Food or food group intake	Reference
	TMAO	Fish	[44]
	4-O-Methylgallic acid Gallic acid Chlorogenic acid	Coffee and tea	[40]
	Citrulline Taurine Isocitrate	Sugar sweetened beverages	[41]
	Resveratrol metabolites	Wine	[42,43]

4. Assessment of user satisfaction

4.1. Health professional–platform user satisfaction

Clinicians using the NUTRISHIELD platform will be asked a series of questions referent to how satisfied they are with the platform, with answers provided on a 4-item Likert scale (neutral answer excluded). The answers provided will be analyzed in order to quantify the user's level of satisfaction. These questions will aim for addressing

- Levels of user-friendliness (not at all user-friendly to extremely user-friendly)
- Levels of advice applicability (not at all applicable to extremely applicable)
- Levels of clinical usefulness (not at all useful to extremely useful)
- Levels of probability of re-using of the platform, or suggesting the platform to a colleague (highly unlikely to highly likely)

4.2. NUTRISHIELD mobile application user satisfaction

Children enrolled in study I will be asked to use the NUTRISHIELD mobile application. They will be asked a series of questions, regarding their levels of app using satisfaction, with answers provided on a 4-item Likert scale (neutral answer excluded). Questions will be adapted from questionnaires used in similar research [45-47]. These questions will aim for addressing

- Enjoying using the app (not at all to very much)
- Probability of suggesting the app to a peer (not at all to very much)
- Satisfaction from technical aspects of the app (i.e. design, network stability, uploading speed etc) (not at all satisfied to highly satisfied)

5. Expectations and role to the project

The present deliverable incorporates knowledge produced under WP 2-6 and WP9. This in essence describes how a theoretical framework designed under NUTRISHIELD translates to clinical practice, and is expected to have great impact on the project.

The novel analyzers designed under NUTRISHIELD are expected to be validated in the context of the clinical studies of WP2. Furthermore, the examination of blood and urine biomarkers reflecting food intake are expected to address the literature gap concerning objective measures of dietary intake and adherence to dietary advice.

Measures of users satisfaction are expected to improve the software developed under WP6, in terms of user-friendliness, interface etc. (as briefly described above).

Last, a major expectation of this project is the evaluation of the effectiveness of the designed trials, in term of producing favorable outcomes for the study population. This is in accordance with NUTRISHIELD's greater objective, which is employing novel methods for ameliorating health outcomes in youth.

6. References

1. Gao Z, Chen S, Sun H, Wen X, Xiang P (2018) Physical Activity in Children's Health and Cognition. Biomed Res Int 2018:8542403. doi:10.1155/2018/8542403
2. Boreham C, Riddoch C (2001) The physical activity, fitness and health of children. Journal of sports sciences 19 (12):915-929. doi:10.1080/026404101317108426
3. Biddle SJ, Asare M (2011) Physical activity and mental health in children and adolescents: a review of reviews. British journal of sports medicine 45 (11):886-895. doi:10.1136/bjsports-2011-090185
4. Te Morenga L, Montez JM (2017) Health effects of saturated and trans-fatty acid intake in children and adolescents: Systematic review and meta-analysis. PloS one 12 (11):e0186672-e0186672. doi:10.1371/journal.pone.0186672
5. Pivina L, Semenova Y, Dosa MD, Dauletyarova M, Bjorklund G (2019) Iron Deficiency, Cognitive Functions, and Neurobehavioral Disorders in Children. Journal of molecular neuroscience : MN. doi:10.1007/s12031-019-01276-1
6. Weydert JA (2014) Vitamin D in Children's Health. Children 1 (2):208-226. doi:10.3390/children1020208
7. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA (1998) Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. The New England journal of medicine 338 (23):1650-1656. doi:10.1056/NEJM199806043382302
8. Dauchet L, Amouyel P, Dallongeville J (2009) Fruits, vegetables and coronary heart disease. Nature reviews Cardiology 6 (9):599-608. doi:10.1038/nrcardio.2009.131
9. Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Laskey MA, Yan L, Prentice A, Ginty F (2006) Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. The American journal of clinical nutrition 83 (6):1420-1428. doi:10.1093/ajcn/83.6.1420
10. Moore LL, Singer MR, Bradlee ML, Djousse L, Proctor MH, Cupples LA, Ellison RC (2005) Intake of fruits, vegetables, and dairy products in early childhood and subsequent blood pressure change. Epidemiology 16 (1):4-11
11. Lanou AJ, Berkow SE, Barnard ND (2005) Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. Pediatrics 115 (3):736-743. doi:10.1542/peds.2004-0548
12. Wang W, Wu Y, Zhang D (2016) Association of dairy products consumption with risk of obesity in children and adults: a meta-analysis of mainly cross-sectional studies. Annals of Epidemiology 26 (12):870-882.e872. doi:https://doi.org/10.1016/j.annepidem.2016.09.005
13. Moreno LA, Bel-Serrat S, Santaliestra-Pasias A, Bueno G (2015) Dairy products, yogurt consumption, and cardiometabolic risk in children and adolescents. Nutrition reviews 73 Suppl 1:8-14. doi:10.1093/nutrit/nuv014
14. Iaccarino Idelson P, Scalfi L, Valerio G (2017) Adherence to the Mediterranean Diet in children and adolescents: A systematic review. Nutrition, Metabolism and Cardiovascular Diseases 27 (4):283-299. doi:https://doi.org/10.1016/j.numecd.2017.01.002
15. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J (2007) Development of a WHO growth reference for school-aged children and adolescents. Bulletin of the World Health Organization 85 (9):660-667

16. Cole TJ, Lobstein T (2012) Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatric obesity 7 (4):284-294. doi:10.1111/j.2047-6310.2012.00064.x
17. Panpanich R, Garner P (2000) Growth monitoring in children. The Cochrane database of systematic reviews (2):CD001443. doi:10.1002/14651858.cd001443
18. Ten S, Maclaren N (2004) Insulin resistance syndrome in children. The Journal of clinical endocrinology and metabolism 89 (6):2526-2539. doi:10.1210/jc.2004-0276
19. Steinbeck KS (2004) Insulin resistance syndrome in children and adolescents: clinical meaning and indication for action. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 28 (7):829-832. doi:10.1038/sj.ijo.0802723
20. Berenson GS (2009) Cardiovascular risk begins in childhood: a time for action. American journal of preventive medicine 37 (1 Suppl):S1-2. doi:10.1016/j.amepre.2009.04.018
21. Cani PD, Everard A (2016) Talking microbes: When gut bacteria interact with diet and host organs. Molecular nutrition & food research 60 (1):58-66. doi:10.1002/mnfr.201500406
22. Kisuse J, La-Ongkham O, Nakphaichit M, Therdtatha P, Momoda R, Tanaka M, Fukuda S, Popluechai S, Kespechara K, Sonomoto K, Lee Y-K, Nitisinprasert S, Nakayama J (2018) Urban Diets Linked to Gut Microbiome and Metabolome Alterations in Children: A Comparative Cross-Sectional Study in Thailand. Frontiers in microbiology 9:1345-1345. doi:10.3389/fmicb.2018.01345
23. Pallister T, Haller T, Thorand B, Altmaier E, Cassidy A, Martin T, Jennings A, Mohney RP, Gieger C, MacGregor A, Kastenmüller G, Metspalu A, Spector TD, Menni C (2017) Metabolites of milk intake: a metabolomic approach in UK twins with findings replicated in two European cohorts. European journal of nutrition 56 (7):2379-2391. doi:10.1007/s00394-016-1278-x
24. Munger LH, Trimigno A, Picone G, Freiburghaus C, Pimentel G, Burton KJ, Pralong FP, Vionnet N, Capozzi F, Badertscher R, Vergeres G (2017) Identification of Urinary Food Intake Biomarkers for Milk, Cheese, and Soy-Based Drink by Untargeted GC-MS and NMR in Healthy Humans. Journal of proteome research 16 (9):3321-3335. doi:10.1021/acs.jproteome.7b00319
25. Hjerpsted JB, Ritz C, Schou SS, Tholstrup T, Dragsted LO (2014) Effect of cheese and butter intake on metabolites in urine using an untargeted metabolomics approach. Metabolomics 10 (6):1176-1185
26. Münger LH, Garcia-Aloy M, Vázquez-Fresno R, Gille D, Rosana ARR, Passerini A, Soria-Florido M-T, Pimentel G, Sajed T, Wishart DS, Andres Lacueva C, Vergères G, Praticò G (2018) Biomarker of food intake for assessing the consumption of dairy and egg products. Genes & nutrition 13:26-26. doi:10.1186/s12263-018-0615-5
27. Gardner CD, Chatterjee LM, Franke AA (2009) Effects of isoflavone supplements vs. soy foods on blood concentrations of genistein and daidzein in adults. The Journal of nutritional biochemistry 20 (3):227-234. doi:10.1016/j.jnutbio.2008.02.008
28. Franke AA, Custer LJ, Hundahl SA (2004) Determinants for urinary and plasma isoflavones in humans after soy intake. Nutrition and cancer 50 (2):141-154. doi:10.1207/s15327914nc5002_3
29. Gibbons H, Michielsen CJR, Rundle M, Frost G, McNulty BA, Nugent AP, Walton J, Flynn A, Gibney MJ, Brennan L (2017) Demonstration of the utility of biomarkers for dietary intake assessment; proline betaine as an example. Molecular nutrition & food research 61 (10). doi:10.1002/mnfr.201700037
30. Nielsen SE, Freese R, Kleemola P, Mutanen M (2002) Flavonoids in human urine as biomarkers for intake of fruits and vegetables. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 11 (5):459-466

31. Penczynski KJ, Remer T, Herder C, Kalhoff H, Rienks J, Markgraf DF, Roden M, Buyken AE (2018) Habitual Flavonoid Intake from Fruit and Vegetables during Adolescence and Serum Lipid Levels in Early Adulthood: A Prospective Analysis. Nutrients 10 (4):488. doi:10.3390/nu10040488
32. Krogholm KS (2011) Flavonoids as fruit and vegetable intake biomarkers. Danmarks Tekniske Universitet, Fødevareinstituttet
Dennmark
33. Ross AB, Aman P, Kamal-Eldin A (2004) Identification of cereal alkylresorcinol metabolites in human urine-potential biomarkers of wholegrain wheat and rye intake. Journal of chromatography B, Analytical technologies in the biomedical and life sciences 809 (1):125-130. doi:10.1016/j.jchromb.2004.06.015
34. Wierzbicka R, Zamaratskaia G, Kamal-Eldin A, Landberg R (2017) Novel urinary alkylresorcinol metabolites as biomarkers of whole grain intake in free-living Swedish adults. Molecular nutrition & food research 61 (7). doi:10.1002/mnfr.201700015
35. Meija L, Krams I, Cauce V, Samaletdin A, Soderholm P, Meija R, Larmane L, Lejnieks A, Lietuvietis V, Adlercreutz H (2015) Alkylresorcinol metabolites in urine and plasma as potential biomarkers of rye and wheat fiber consumption in prostate cancer patients and controls. Nutrition and cancer 67 (2):258-265. doi:10.1080/01635581.2015.989371
36. Mitry P, Wawro N, Rohrmann S, Giesbertz P, Daniel H, Linseisen J (2018) Plasma concentrations of anserine, carnosine and pi-methylhistidine as biomarkers of habitual meat consumption. European journal of clinical nutrition. doi:10.1038/s41430-018-0248-1
37. Freisling H, Assi N, Slimani N, Keski-Rahkonen P, Ferrari P, Zamora-Ros R, Rinaldi S, Cheung W, Scalbert A, Kaaks R, Kühn T, Floegel A, Boeing H, Mancini F, Boutron-Ruault M-C, Baglietto L, Naska A, Trichopoulou A, Orfanos P, Frost G, Rundle M, Cross AJ, Carr E, Gibbons H, Brennan L, Pala V, Panico S, Sacerdote C, Palli D, Tumino R (2017) A metabolomic study of biomarkers of meat and fish intake. The American journal of clinical nutrition 105 (3):600-608. doi:10.3945/ajcn.116.146639
38. Cross AJ, Major JM, Sinha R (2011) Urinary biomarkers of meat consumption. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 20 (6):1107-1111. doi:10.1158/1055-9965.EPI-11-0048
39. Myint T, Fraser GE, Lindsted KD, Knutsen SF, Hubbard RW, Bennett HW (2000) Urinary 1-methylhistidine is a marker of meat consumption in Black and in White California Seventh-day Adventists. American journal of epidemiology 152 (8):752-755
40. Hodgson JM, Chan SY, Puddey IB, Devine A, Wattanapenpaiboon N, Wahlqvist ML, Lukito W, Burke V, Ward NC, Prince RL, Croft KD (2004) Phenolic acid metabolites as biomarkers for tea- and coffee-derived polyphenol exposure in human subjects. The British journal of nutrition 91 (2):301-306. doi:10.1079/bjn20031046
41. Gibbons H, McNulty BA, Nugent AP, Walton J, Flynn A, Gibney MJ, Brennan L (2015) A metabolomics approach to the identification of biomarkers of sugar-sweetened beverage intake. The American journal of clinical nutrition 101 (3):471-477. doi:10.3945/ajcn.114.095604
42. Zamora-Ros R, Urpi-Sarda M, Lamuela-Raventos RM, Estruch R, Vazquez-Agell M, Serrano-Martinez M, Jaeger W, Andres-Lacueva C (2006) Diagnostic performance of urinary resveratrol metabolites as a biomarker of moderate wine consumption. Clinical chemistry 52 (7):1373-1380. doi:10.1373/clinchem.2005.065870
43. Zamora-Ros R, Urpi-Sarda M, Lamuela-Raventos RM, Estruch R, Martinez-Gonzalez MA, Bullo M, Aros F, Cherubini A, Andres-Lacueva C (2009) Resveratrol metabolites in urine as a biomarker of wine

intake in free-living subjects: The PREDIMED Study. Free radical biology & medicine 46 (12):1562-1566. doi:10.1016/j.freeradbiomed.2008.12.023

44. Cho CE, Taesuwan S, Malysheva OV, Bender E, Tulchinsky NF, Yan J, Sutter JL, Caudill MA (2017) Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. Molecular nutrition & food research 61 (1):1600324
45. Stoll R, Pina A, Gary K, Amresh A (2017) Usability of a Smartphone Application to Support the Prevention and Early Intervention of Anxiety in Youth. Cognitive and behavioral practice 24 (4):393-404. doi:10.1016/j.cbpra.2016.11.002
46. Lund AM (2001) Measuring usability with the use questionnaire12. Usability interface 8 (2):3-6
47. Liew MS, Zhang J, See J, Ong YL (2019) Usability Challenges for Health and Wellness Mobile Apps: Mixed-Methods Study Among mHealth Experts and Consumers. JMIR Mhealth Uhealth 7 (1):e12160. doi:10.2196/12160