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Nutritional metabolomics in assessing dietary patterns: A review

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ABSTRACT

Metabolomics is a stream of study that deals with small metabolites or a set of tiny molecules. Metabolomics has been widely adapted in toxicology and pharmacology. However, they are yet to be utilized in nutrition and food science. Nutrition metabolomics can be defined as the study of metabolites generated from dietary compounds after host metabolism on the consumption of a certain food item. The quality and quantity of food consumed determine the health of a person. In recent years metabolomics has gained importance for assessing nutrition and dietary intake. This is because it has provided a novel strategy to discover new metabolomic biomarkers that would help to understand the association between genetic makeup and nutrition. There will be associated opportunities and challenges in nutritional metabolomics which can help to determine the fate of personalized diets. The target of this paper is to discuss various forms of study designs, measurement techniques, and statistical approaches for identifying metabolites that are associated with dietary patterns and dietary intakes.

Keywords: *Nutritional metabolomics; NMR; dietary patterns; interventional trials; PLS-DA.*

1. INTRODUCTION

With advancements in technologies, high-throughput screening techniques are advancing the fields of molecular biology. In the past few years, researchers have focused on creating a system that integrates several scientific fields of study to link existing knowledge with novel strategies. Metabolite concentration monitoring assists in the analysis and assessment of physiological processes in the body. Metabolomics permits the measurement of metabolic effects of nutrients. It is important to identify the correct metabolomic techniques and biomarkers to obtain the correct result for the study. The aim of nutrition metabolomics is to combine the knowledge of metabolomics and nutrition in order to achieve a better understanding of the impact of exogenous elements on biological metabolic regulations (Brennan, 2017a; Banerjee and Jaiswal, 2019). This would in turn help in designing a technique that would help in the personalization of diets on the basis of the genetic makeup of an individual. This would further help in improved management and prevention of diseases. There are reports in which it has been investigated that the dietary treatments with different fruits or part of the plants were used for the treatment of the disease (Konda et al., 2021). The biomolecules that play an important role in the cure of the treatment of the numerous complications come from the metabolites and can be considered as nutritional metabolites (Konda et al., 2020a). The nutritional metabolites of different nutrient resources are also known to prevent various complications of disease and provide supplements for proper nutrition (Konda et al., 2020b; Jaiswal et al., 2020). There are several factors that influence the metabolome such as age, genetic factors, diseases, drugs, nutrition, and environment.

It is possible to obtain an entire profile of small metabolites from different biological samples such as blood, urine, saliva, and even stool. This would provide a comprehensive picture of an individual's dietary intake (O'Gorman and Brennan, 2017). Nutrition metabolomics would provide further knowledge of different metabolic pathways involved in metabolism.

2. TECHNIQUES AND APPROACHES USED IN METABOLOMICS STUDIES

Metabolomics can be described as the systematic study and analysis of biochemical compounds with or small molecular weight which are generally found in biological samples such as plasma urine serum blood and stool. Some of the most important and widely-used biofluids are blood plasma, urine, and serum.

Urine has both an advantage and a disadvantage. One of the advantages of urine is that most of the metabolites found in urine are excreted by the body faster than any other biofluids (Riekeberg and Powers, 2017). However, urine contains a greater concentration of non-nutrient compounds and non-metabolites

which can make the analysis complicated. The metabolites excreted by urine are often used for the analysis of commonly consumed foods, such as proline betaine. Blood majorly contains high content of active metabolites. Metabolites present in plasma are generally lipid-soluble (Horner et al., 2020).

The two most widely used techniques in metabolomics are "proton nuclear magnetic resonance (NMR) spectroscopy" and "mass spectrometry". Although "inductively coupled plasma mass spectrometry" is also used in certain cases, however, the former two are most popular. According to Konz et al., (2017), many researchers have used a targeted approach where they analyze a subset of a predetermined metabolite. Targeted approach-based techniques are comparatively affordable and it depends on a mechanistic approach where predefined metabolites are targeted for detecting biological pathways of clinical importance. However, there are certain drawbacks as it is expensive, requires a complex set of data, and utilizes complex analytical techniques. There is an extensive database for human metabolome that contains almost 7,000 "fully annotated human metabolites" and it is known as the "Human Metabolome database" (Wishart et al., 2018).

3. STUDY DESIGNS

Study designs can be divided into two categories- Observational and Interventional.
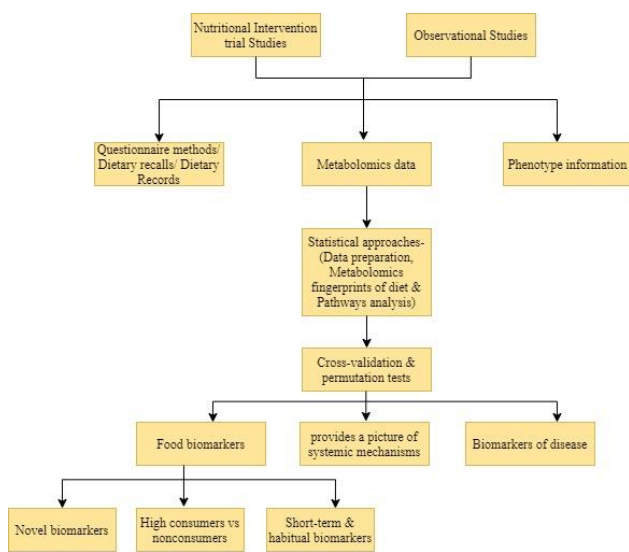

Figure 1. Flowchart on different approaches of nutritional metabolomics.

Observational Studies

Metabolomics can help to identify a number of nutritional biomarkers which can help to assess the dietary intake of a person. Multiple metabolites can be identified in a single test which can provide precise and accurate dietary assessment if it can be properly combined with a "Food frequency questionnaire (FFQ)" (Kim and Rebholz, 2021). In this, subjects maintain a record of all the items and measurements they consumed over the time of the study. This provides general information on the regular diet of the subjects such as the quality of food consumed every meal, frequency of consumption, most commonly consumed items, food items that are not included in the diet (Shibutami et al., 2021). These objective biomarkers act as criteria that are equally important for understanding the habitual intake of the subjects. This helps to associate the metabolomic studies with the consumption and dietary pattern of the subjects.

However, it should be remembered that most food items are correlated and may not be specifically associated with an identified biomarker. This can be explained with an example- Vitamin A, retinoids, and carotenoids are found in a wide variety of fruits and vegetables, therefore, they have to be used as a generic biomarker for a set of fruits and vegetables. It cannot be specifically associated with a single fruit or vegetable (Scalbert et al., 2017). Some studies have used fragmentary designs where they compare non-consumers and consumers, while others have adopted longitudinal variations observed in metabolite concentrations and their relation to dietary and health patterns. One of the important criteria of observational studies is that the sample size of the subjects is really small and is not replicated. This is because the analysis of such studies is yet to be homogenized and standardized (Zhou et al., 2019). Some of the areas of differences and risks are different platforms used for analysis, storage of biofluids, techniques for quantification of metabolites, and biases of subjects while maintaining the record. Evaluation of biological variability and metabolite stability of a population is equally important.

Interventional Studies

One of the key importance of metabolomics is that it can be used to identify diet-based novel biomarkers. The "intersubject metabolic variations" need to be accounted for the purpose. The complexity lies in the quantification of subtle metabolic modulations in opposition to the small doses of bioactive nutrients (Münger et al., 2018). One of the methods used in nutritional metabolomics is intervention feeding trials, where subjects are given a specific set of foods to consume as single or multiple meals, ranging from a week to months. These can be either short or long-term as demanded by the research interest (Bazanella et al., 2017). In long-term cases, cross-over studies are favored because respondents can have control and intersubject variations can be effectively handled. Biofluids can be obtained either or both, before and after the specified meals. Other than that, biofluids are obtained at the beginning and termination of the intervention trials. Results obtained from small-term studies need to be replicated and validated with a long-term study. Some of the important considerations include precise and accurate measurement of "dietary exposures" (Rådjursöga et al., 2019). Other than that nutritional metabolomics demands identification of objective markers of dietary interventions and their related compliance.

4. DATA INTERPRETATION

Screening of high-throughput and complicated data can be challenging without proper technology. Now, advanced computational and bioinformatics tools are used for the analysis of data related to nutritional metabolomics. Some of the important steps of data analysis include data preparation, data reduction, normalization, and final interpretation.

One of the first steps of metabolomics is to gather the raw data from the biofluid samples and develop a metabolomic profile. The data generally undergoes a set of standardized techniques of pre-processing, alignment, normalization, and signal correction, before the analysis (O'Gorman and Brennan, 2017). Pre-processing is conducted through mass spectrometry where aspects like peak detection, "retention time alignment", peak matching, filling, and integration are carried out (Brennan, 2017b). Data alignment can be described as the method of "matching retention times and peaks to the standardized values" for different profile samples. Pooled study samples and internal standards can be used to homogenize data across the data sets (Weimer and Slupsky, 2013). Sometimes computational tools are used to compare the obtained peaks to

previous studies to fund out if there are any variations in the peaks of the biosamples. Repeatability filters are used to carry out quality control at the current phase. According to Carneiro et al., (2019), when using NMR spectroscopy, peak shift and peak overlap are included in the pre-processing phase. Peak shifts are accounted for by utilizing grouping or "binning" of structure-based correlated metabolite signals (Haslauer et al., 2021). Some of the other important phases of data preparation include outlier removal, scaling, and batch normalization. Batch normalization of spectrometric signals is crucial as it eliminates undesired variations (Misra, 2020). Annotation is followed by the application of statistical tools for the analysis of the data. Comparison of the obtained data with standardized databases is important for eliminating "putative annotation" (Wolthuis et al., 2020). In cases of missing values, either respondent with missing metabolites should be eliminated from the study or replaced with a non-zero value while maintaining the structure, as advised by Guasch-Ferre et al., (2018).

EXPERIMENTAL DESIGN	SAMPLE PREP	DATA ACQUISITION	DATA PROCESSING	STATISTICAL ANALYSIS
- Research Questions - Metabolomics Approaches	- Bio-samples/ tissues/ biofluids - Extraction	- LC/MS - GC/MS - NMR	- Peak detection - Baseline correction - Peak alignment - Normalization - Deconvolution	- Univariate analysis - Multivariate analysis - Pathway analysis

Figure 2. Overview of the stages of metabolomics (Source: O'Gorman, & Brennan, 2017).

5. STATISTICAL APPROACHES

Bioinformatics and statistical tools are applied for recovering and interpreting high-dimensional datasets. Data with a high degree of similarity are divided into groups by the clustering method such as PCA, unsupervised clustering algorithms, and PLS-DA (Tebani and Bekri, 2019). In these cases, a set of metabolites is created irrespective of a hypothesis on metabolic pathways and their relation to dietary information. In the case of an unsupervised PCA, a linear transformation is derived with retains the variance of the original data (Zhang et al., 2017). During the process, it minimizes intra-group variation and maximizes inter-group variations. However, in the case of PLS-DA, the covariance between predictor and outcome scores is maximized as it accounts for the correlation between outcome and metabolomics data (Luong et al., 2019). In order to classify metabolomic profiles according to a set category such as dietary patterns discriminant analysis is carried out through "Partial Least Square Analysis". Other than this "supervised multivariate analysis" is also used for nutritional metabolomics. Metabolic data which are not associated with the predetermined classes are

generally filtered out by PLS-DA (Khorraminezhad et al., 2020). The O-PLS-DA technique provides a better interpretation of metabolomics data due to the separation of the structured noise from the variation common of the matrices. O-PLS-DA model can be used to develop a metabolomic profile that can be predictive of the dietary pattern (Mora-Ortiz et al., 2019). The external factors that are not linearly associated with it such as age, gender, and batch are also captured.

These statistical methods also require validation through cross-validation, external validation, and permutation testing (Ulaszewska et al., 2019). It is essential to prevent false-positive discoveries to ensure the authenticity of the data. It is often recommended that "false-positive discovery rate" and Bonferroni adjustment account for several crucial comparison testing, such as CIs, P values, adjustment of covariates, and effect size (Zhu et al., 2019, Gibney et al., 2005).

6. PATHWAY ANALYSIS

There are some metabolic databases that have been developed in the past few years such as MetaCyc, BIGG, and KEGG families (Pham et al., 2019). These can be used to elucidate the metabolic pathways, integrated untargeted and targeted metabolomics. In order to derive metabolic sets, "semi-supervised pathway analysis" is based on gene enrichment analysis (Pomyen et al., 2020). "Agnostic network models" can also be used for combining crucial metabolites identified from both targeted and untargeted approaches to authenticate metabolomic signatures (Guasch-Ferre et al., 2018). Integration of data into metabolic pathways can be achieved through combining algorithms and correlation networks for active subnetworks detection. These tools can help with the visualization of metabolic pathways for analyzing the metabolic networks associated with dietary patterns.

Most studies in nutritional metabolomics are related to the recognition of dietary biomarkers for specific food groups and foods, with little work on specific nutrients. Some of the commonly studied food items are chocolate, nuts, coffee, wine, meat, cereals, fruits, fish, and vegetables.

7. CONCLUSIONS

Diet is an important factor that determines the well-being of a person. Nutritional research is been advancing with new techniques, methods, and screening tools. It is helping to obtain valuable insight on the processes and elements that are related to dietary patterns and dietary exposure. Metabolomics has turned out to be a valuable tool in discovering important dietary biomarkers. The application of metabolomics has enhanced and expanded the ability to understand and study validated biomarkers. The validated biomarkers need to be analyzed using specific and standardized techniques to translate the dietary patterns into practice.

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9. DECLARATION

The author declares no conflict of interest.

10. REFERENCES

Banerjee, I., & Jaiswal, K. K. (2019). The role of the Klotho protein in the function of aging and neurodegenerative disorders. Octa J. Biosci, 7, 113-118.

Bazanella, M., Maier, T. V., Clavel, T., Lagkouvardos, I., Lucio, M., Maldonado-Gòmez, M. X., ... & Haller, D. (2017). Randomized controlled trial on the impact of early-life intervention with bifidobacteria on the healthy infant fecal microbiota and metabolome. The American journal of clinical nutrition, 106(5), 1274-1286.

Brennan, L. (2017a). The nutritional metabolomics crossroads: how to ensure success for dietary biomarkers.

Brennan, L. (2017b). Metabolomics: a tool to aid dietary assessment in nutrition. Current Opinion in Food Science, 16, 96-99.

Carneiro, G., Radcenco, A. L., Evaristo, J., & Monnerat, G. (2019). Novel strategies for clinical investigation and biomarker discovery: a guide to applied metabolomics. Hormone molecular biology and clinical investigation, 38(3).

Gibney, M. J., Walsh, M., Brennan, L., Roche, H. M., German, B., & Van Ommen, B. (2005). Metabolomics in human nutrition: opportunities and challenges–. The American journal of clinical nutrition, 82(3), 497-503.

Guasch-Ferre, M., Bhupathiraju, S. N., & Hu, F. B. (2018). Use of metabolomics in improving assessment of dietary intake. Clinical chemistry, 64(1), 82-98.

Haslauer, K. E., Schmitt-Kopplin, P., & Heinzmann, S. S. (2021). Data Processing Optimization in Untargeted Metabolomics of Urine Using Voigt Lineshape Model Non-Linear Regression Analysis. Metabolites, 11(5), 285.

Horner, K., Hopkins, M., Finlayson, G., Gibbons, C., & Brennan, L. (2020). Biomarkers of appetite: is there a potential role for metabolomics? Nutrition research reviews, 33(2), 271-286.

Jaiswal, K. K., Banerjee, I., & Mayookha, V. P. (2020). Recent trends in the development and diversification of sericulture natural products for innovative and sustainable applications. Bioresource Technology Reports, 100614.

Khorraminezhad, L., Leclercq, M., Droit, A., Bilodeau, J. F., & Rudkowska, I. (2020). Statistical and machine-learning analyses in nutritional genomics studies. Nutrients, 12(10), 3140.

Kim, H., & Rebholz, C. M. (2021). Metabolomic Biomarkers of Healthy Dietary Patterns and Cardiovascular Outcomes. Current atherosclerosis reports, 23(6), 1-12.

Konda, P. Y., Chennupati, V., Dasari, S., Sharma, N., Muthulingam, M., Ramakrishnan, R., ... & Jaiswal, K. K. (2021). Ethno-pharmacological insulin signaling induction of aqueous extract of Syzygium paniculatum fruits in a high-fat diet induced hepatic insulin resistance. Journal of ethnopharmacology, 268, 113576.

Konda, P. Y., Egi, J. Y., Dasari, S., Katepogu, R., Jaiswal, K. K., & Nagarajan, P. (2020b). Ameliorative effects of Mentha aquatica on diabetic and nephroprotective potential activities in STZ-induced renal injury. Comparative Clinical Pathology, 29(1), 189-199.

Konda, P. Y., Poondla, V., Jaiswal, K. K., Dasari, S., Uyyala, R., Surtineni, V. P., ... & Natesan, V. (2020a). Pathophysiology of high fat diet induced obesity: impact of

probiotic banana juice on obesity associated complications and hepatosteatosis. Scientific Reports, 10(1), 1-17.

Konz, T., Migliavacca, E., Dayon, L., Bowman, G., Oikonomidi, A., Popp, J., & Rezzi, S. (2017). ICP-MS/MS-based ionomics: a validated methodology to investigate the biological variability of the human ionome. Journal of proteome research, 16(5), 2080-2090.

Luong, D. V., Tam, N. Q., Xuan, D. T. T., & Tai, N. T. (2019). NMR based metabolomic approach for evaluation of Vietnamese honey. Vietnam Journal of Chemistry, 57(6), 712-716.

Misra, B. B. (2020). Data normalization strategies in metabolomics: Current challenges, approaches, and tools. European Journal of Mass Spectrometry, 26(3), 165-174.

Mora-Ortiz, M., Ramos, P. N., Oregioni, A., & Claus, S. P. (2019). NMR metabolomics identifies over 60 biomarkers associated with Type II Diabetes impairment in db/db mice. Metabolomics, 15(6), 1-16.

Münger, L. H., Trimigno, A., Picone, G., Freiburghaus, C., Pimentel, G., Burton, K. J., ... & Vergères, 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.

O'Gorman, A., & Brennan, L. (2017). The role of metabolomics in determination of new dietary biomarkers. Proceedings of the Nutrition Society, 76(3), 295-302.

Pham, N., van Heck, R. G., van Dam, J. C., Schaap, P. J., Saccenti, E., & Suarez-Diez, M. (2019). Consistency, inconsistency, and ambiguity of metabolite names in biochemical databases used for genome-scale metabolic modelling. Metabolites, 9(2), 28.

Pomyen, Y., Wanichthanarak, K., Poungsombat, P., Fahrmann, J., Grapov, D., & Khoomrung, S. (2020). Deep metabolome: Applications of deep learning in metabolomics. Computational and Structural Biotechnology Journal.

Rådjursöga, M., Lindqvist, H. M., Pedersen, A., Karlsson, G. B., Malmodin, D., Brunius, C., ... & Winkvist, A. (2019). The 1 H NMR serum metabolomics response to a two-meal challenge: a cross-over dietary intervention study in healthy human volunteers. Nutrition journal, 18(1), 1-12.

Riekeberg, E., & Powers, R. (2017). New frontiers in metabolomics: from measurement to insight. F1000Research, 6.

Scalbert, A., Rothwell, J. A., Keski-Rahkonen, P., & Neveu, V. (2017). The food metabolome and dietary biomarkers. In Advances in the assessment of dietary intake (pp. 259-282). CRC Press.

Shibutami, E., Ishii, R., Harada, S., Kurihara, A., Kuwabara, K., Kato, S., ... & Takebayashi, T. (2021). Charged metabolite biomarkers of food intake assessed via plasma metabolomics in a population-based observational study in Japan. PloS one, 16(2), e0246456.

Tebani, A., & Bekri, S. (2019). Paving the way to precision nutrition through metabolomics. Frontiers in nutrition, 6, 41.

Ulaszewska, M. M., Weinert, C. H., Trimigno, A., Portmann, R., Andres Lacueva, C., Badertscher, R., ... & Vergères, G. (2019). Nutrimetabolomics: an integrative action for metabolomic analyses in human nutritional studies. Molecular nutrition & food research, 63(1), 1800384.

Weimer, B. C., & Slupsky, C. (Eds.). (2013). Metabolomics in food and nutrition. Elsevier.

Wishart, D. S., Feunang, Y. D., Marcu, A., Guo, A. C., Liang, K., Vázquez-Fresno, R., ... & Scalbert, A. (2018). HMDB 4.0: the human metabolome database for 2018. Nucleic acids research, 46(D1), D608-D617.

Wolthuis, J. C., Magnusdottir, S., Pras-Raves, M., Moshiri, M., Jans, J. J., Burgering, B., ... & de Ridder, J. (2020). MetaboShiny: interactive analysis and metabolite annotation of mass spectrometry-based metabolomics data. Metabolomics, 16(9), 1-6.

Zhang, A., Sun, H., & Wang, X. (2017). Emerging role and recent applications of metabolomics biomarkers in obesity disease research. Rsc Advances, 7(25), 14966-14973.

Zhou, Y. F., Luo, B. A., & Qin, L. L. (2019). The association between vitamin D deficiency and community-acquired pneumonia: A meta-analysis of observational studies. Medicine, 98(38).

Zhu, Z., Camargo Jr, C. A., & Hasegawa, K. (2019). Metabolomics in the prevention and management of asthma. Expert review of respiratory medicine, 13(12), 1135-1138.