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Review

The emergence of proton nuclear magnetic resonance metabolomics in the cardiovascular arena as viewed from a clinical perspective

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ABSTRACT

The ability to phenotype metabolic profiles in serum has increased substantially in recent years with the advent of metabolomics. Metabolomics is the study of the metabolome, defined as those molecules with an atomic mass less than 1.5 kDa. There are two main metabolomics methods: mass spectrometry (MS) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, each with its respective benefits and limitations. MS has greater sensitivity and so can detect many more metabolites. However, its cost (especially when heavy labelled internal standards are required for absolute quantitation) and quality control is sub-optimal for large cohorts. ^{1}H NMR is less sensitive but sample preparation is generally faster and analysis times shorter, resulting in markedly lower analysis costs. ^{1}H NMR is robust, reproducible and can provide absolute quantitation of many metabolites. Of particular relevance to cardiometabolic disease is the ability of ^{1}H NMR to provide detailed quantitative data on amino acids, fatty acids and other metabolites as well as lipoprotein subparticle concentrations and size. Early epidemiological studies suggest promise, however, this is an emerging field and more data is required before we can determine the clinical utility of these measures to improve disease prediction and treatment.

This review describes the theoretical basis of ^{1}H NMR; compares MS and ^{1}H NMR and provides a tabular overview of recent ^{1}H NMR-based research findings in the atherosclerosis field, describing the design and scope of studies conducted to date. ^{1}H NMR metabolomics-CVD related research is emerging, however further large, robustly conducted prospective, genetic and intervention studies are needed to advance research on CVD risk prediction and to identify causal pathways amenable to intervention.

Contents

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1. Introduction

The metabolome is the entire small molecule (metabolite) complement of a system. Metabolites are generally defined as having an atomic mass of less than 1.5 kDa [1,2]. In humans, these metabolites can be exogenous (e.g. dietary or drug related), endogenous (substrates, intermediates and final products of chemical reactions), and derived from the effect of the microbiome. Metabolites include carbohydrates, peptides, lipids, nucleotides, amino acids, organic acids and many other classes of small molecule [3,4].

Metabolomics is the use of analytical chemistry methods combined with chemometrics for the study of the metabolome. Chemometrics, in turn, is the application of statistical and computational methods to extract data from experimentally derived spectra. The two most commonly used methods of probing the metabolome are: mass spectrometry (MS) and proton nuclear magnetic resonance (^{1}H NMR).

There are two ways of quantifying the metabolites in a metabolomics experiment, termed absolute and relative quantitation [5]. For relative quantitation the (normalised) instrument response to the metabolite(s) is used to obtain a measure of that metabolite which can be compared within that cohort or batch [5]. However, as these are not in SI units, it is difficult to compare groups to other studies or, within the cohort, fully understand the clinical importance of results. The second way of quantifying metabolites, absolute quantitation, is more stringent [5]: involving calibrators and numerous isotopically-labelled internal standards (IS) (depending on the method) [6].

There are two main methodological strategies for probing the metabolome: targeted and untargeted (global) methods [2,7]. In targeted metabolomics a pre-defined subset of metabolites are chosen and a particular analytical method optimised for that subset is used [7]. In non-targeted metabolomics, the aim is to identify and quantify as many metabolites as possible [8,9]. However, due to the diverse nature of metabolites in terms of their physio-chemical properties and dynamic range (ratio of highest versus lowest concentration: e.g. pM to mM) there is no single method that can detect all metabolites [3,8]. Targeted methods report fewer metabolites and are more likely to be hypothesis driven.

Cardiovascular disease (CVD) remains the leading cause of death worldwide [10]. Hypertension, smoking, diabetes mellitus and dyslipidaemia are major risk factors for CVD [11] and are incorporated into risk scores. Such scores are important in assessing treatment needs for primary prevention and are widely used. However, such scores are not perfect and researchers are continually working to improve these scores [12]. It is hoped that methods that probe the metabolome and lipoprotein profile could potentially be used to identify novel biomarkers or pathways for atherosclerosis, improve clinical prediction of CVD, and investigate the metabolic consequences of specific therapies or interventions [8,11,13,14].

This review will briefly outline the key methodological principles of ^{1}H NMR. We focus on ^{1}H NMR because of the recent advances with this method; its markedly lower cost in comparison to MS, resulting in an increasing number of clinically relevant studies using this technique, and the potential for clinical application, already being realised to some extent in the USA [9,15,16]. To illustrate the potential of ^{1}H NMR technology we will review some early gains in the cardiometabolic arena from ^{1}H-NMR-based studies. We also highlight the requirements that need to be met before ^{1}H NMR is widely adopted in epidemiological research and, ultimately, applied to routine clinical care. Finally, we briefly describe the benefits and limitations of ^{1}H NMR, making reference to MS as a comparator method. In so doing, we suggest the two methods provide complementary, rather than competing, methodologies.

2. Proton ^{1}H NMR

2.1. The theoretical basis of proton ^{1}H NMR

^{1}H NMR spectroscopy is a technique that exploits the magnetic properties of protons in order to obtain information about the structure of a molecule, and hence its identity [17]. The sample is placed in a strong magnetic field and electromagnetic radiation, in the form of radiofrequency pulses, is used to excite the protons (Fig. 1). As the protons relax back to equilibrium the energy is recorded as an oscillating electromagnetic signal, called the free induction decay (FID). This is analogous to a number of bells ringing out after they have been simultaneously struck – each frequency of each bell will be overlaid and they will decay together. This complex waveform (intensity versus time) is normally Fourier Transformed (mathematically deconvoluted) in order to produce a spectrum of intensity versus frequency [18]. This is analogous to separating out the individual frequencies sounded by each bell, identifying what all those frequencies were and how loud each one was.

The data are represented as a spectrum of peaks with chemical shift (δ), in parts per million (ppm), along the x-axis and intensity along the y-axis. The chemical shift is the resonant frequency of the nucleus compared to the nucleus of an internal standard (IS), normally tetramethylsilane (TMS) or a related compound. The distance (in ppm) between the resonant frequency observed and the TMS signal depends on the chemical environment of the proton, i.e. the molecular structure. Different protons in different parts of the molecule have a different chemical shift and molecules give a specific pattern of peaks, in terms of both the chemical shift and the intensities of those peaks (Fig. 2). Quantitative ^{1}H NMR (qNMR) is also achieved by comparison to the intensity of this reference peak (normally added to the sample at a known concentration), after taking into account the number of protons contributing to each peak.

^{1}H NMR is a versatile method, with different pulse programs available for optimisation of large or small molecules by enhancing or attenuating different signals (Fig. 2). For example, the