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Biofluid ^{1}H NMR-based metabonomic techniques in nutrition research — metabolic effects of dietary isoflavones in humans

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Abstract

A metabonomic approach to nutrition research may provide an insight into in vivo mechanisms of action following nutritional intervention. This approach was applied to investigate changes in the ^{1}H NMR spectral profile of urine collected from controlled dietary intervention studies conducted in premenopausal women before and following soy or miso consumption. The aim of the study was to identify the biochemical effects of a diet rich in soy isoflavones, phytochemicals which are receiving significant attention because of their potential importance to human health and wide bioactivity in vitro. By applying various chemometric techniques to the data the biochemical effects of conjugated and unconjugated isoflavones were determined. The biochemical changes observed suggest that soy isoflavone ingestion had significant effects on several metabolic pathways associated with osmolyte fluctuation and energy metabolism. These biochemical changes were more significant following ingestion of the unconjugated soy isoflavone (miso) diet suggesting that the chemical composition of the isoflavones present in soy-based foods may have an effect on their biological efficacy in vivo. This study describes a novel application for ^{1}H NMR analysis by determining subtle differences in biochemical profiles following dietary intervention and providing further insight into the mechanisms of action of phytochemicals in vivo.

1. Introduction

Interest in the relative importance of phytochemicals to human health has increased dramatically over the last decade with particular interest in relation to the class of compounds known as the phytoestrogens, which embody several groups of compounds including isoflavones, lignans, coumestans and prenyl flavonoids [1]. These compounds exert a wide range of hormonal and nonhormonal activities in animals or in vitro, and these suggest plausible mechanisms for potential physiological effects of diets rich in these compounds in humans [2]. In addition, experimental and epidemiological data are available to support the concept that isoflavone-rich diets exert physiological effects and preliminary human studies suggest a potential role in hormone-dependent diseases [1].

The biological actions of isoflavones are complex, and their ultimate cellular actions are determined by many factors including the relative levels of oestrogen receptor (ER) α and β, the diverse mixture of coactivators and corepressors present in any given cell type, and the nature of the response elements with which the receptors interact on the oestrogen-regulated genes [3]. It is therefore not surprising that the resulting effects observed from available in vitro and in vivo experiments are inconsistent since the biological effects vary

Abbreviations: AMIX program, analysis of mixtures; d, chemical shift; COSY, correlation spectroscopy; DMA, dimethylamine; DMG, dimethylglycine; ER, oestrogen receptor; FID, free induction decay; FMO3, flavin-containing monooxygenase; 3FT, Fourier transform; OSC, orthogonal signal correction; 2-OG, 2-oxoglutarate; ^{1}H NMR, proton nuclear magnetic resonance; PABA, *para* amino benzoic acid; PCA, principal component analysis; TCA, tricarboxylic acid cycle; TMA, trimethyamines; TMAO, trimethylamine-N-oxide; TSP, 3(trimethylsilyl)propionic-(2,2,3,3-d_4) acid.

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depending on the phytoestrogen compound studied, cell line used, species and tissue under examination. Although the reported oestrogenic potency of isoflavones is weak, 100–1000 times less compared with 17-β-oestradiol, their biological potential cannot be ignored, as typical circulating levels of isoflavones can exceed endogenous estradiol concentrations by 10,000-fold following consumption of a diet containing soy foods [4]. The isoflavone genistein is a potent agonist for ERβ, and the divergent transcriptional activities of oestrogens and isoflavones result not only from their different binding affinities but also from differences in their ability to recruit coregulators and trigger transcriptional functions of ERα and ERβ [5]. Numerous other biological effects independent of the ER (e.g., antioxidant capacity, antiproliferative and antiangiogenic effects) have been ascribed to phytoestrogens and many of these mechanisms are common to other plant phenolics [1,2].

In order to identify the in vivo biochemical effects of dietary phytoestrogens in humans, a metabonomic approach based on ^{1}H NMR spectroscopy of human urine was applied to investigate the biochemical effects of a diet rich in phytoestrogens in premenopausal women. Metabonomics is defined as "The quantitative measurement of time-related multiparametric metabolic responses of an intact living system to pathophysiological stimuli or genetic modification and thus provides a systems approach to understanding metabolic variation in complex multicellular organisms. Successful applications of this metabonomic approach in toxicity screening, drug metabolism and functional genomics have been documented, but these studies have mainly focused on the analysis of data from animals rather than humans [6], due to the greater control of condition in such studies to reduce extrinsic variability. The variability in the composition of urine is significant as the profile is affected by numerous environmental factors, for example, diurnal variation and estrus cycle [7]. In addition, with human data intersubject variation in metabolism is generally significantly greater than that observed in laboratory animal models because of their greater diversity in genetic and environmental factors. One method of minimising some of these environmental conditions in humans is by conducting carefully controlled dietary intervention studies, where each subject consumes a defined diet and participates in both a control and test intervention study period. Following dietary intervention, sophisticated techniques, like metabonomics, are required to determine accurately the subtle metabolic effects related to dietary change. In the current study, the availability of 24-h urine samples from such a controlled intervention study enabled the evaluation and determination of the suitability of this in vivo approach for the assessment of biochemical effects in vivo following a nutrition intervention in a human population.

Although in the past metabonomic techniques using ^{1}H NMR spectroscopy have previously been applied in toxicological studies [6], their application to human data is to date limited. In the area of nutrition research, a metabonomic approach for urine analysis has never previously been reported, although such an approach is complementary to the application of other proteomic and genomic tools to further develop functional biomarkers to determine subtle differences in biochemical profiles following dietary intervention.

2. Experimental protocol and sample collection

Complete 24-h urine samples were obtained as previously described by Cassidy et al. [8,9]. Healthy nonvegetarian women (21–29 years of age) were enrolled in the study. All women had regular, ovulatory menstrual cycles and had taken no medication for $\geq$6 months before starting the study. For two complete menstrual cycles, diets and physical activities were closely monitored and controlled. Basal metabolic rate was estimated from body weight, and the energy intake necessary to maintain a constant body weight throughout the study was calculated, assuming a ratio of total energy intake to basal metabolic rate of 1.4:1.0, to ensure that any observed changes in energy metabolism were not due to changes in the dietary energy content.

The first complete menstrual cycle served as a control period during which time each subject consumed a constant daily diet of non-soy-containing foods provided by a metabolic kitchen [1,2]. During the second month and starting on the first day of menses, appropriate modifications were made to the basal diet to maintain the amounts of micronutrients and nonstarch polysaccharides with the addition of either 60 g/day (dry weight) textured vegetable protein/day (n=6) or 50 g miso/day (n=3) to meals. These two foods were chosen as they contain isoflavones in different chemical forms conjugated (glucosides) or unconjugated (aglycones), respectively. All meals were prepared in advance, accurately weighted and deep-frozen until required. All frozen and canned foods were of the same batch to minimize interbatch variability, and bread that contained no soy flour was specially prepared for the study. The same batch of soy protein was used throughout the study period.

During each diet period 24-h urine samples were obtained at 3-day intervals. Urine volumes were recorded and aliquots taken and stored at −20°C. As an exogenous marker to monitor compliance with the dietary intervention, and to ensure completeness of the 24-h urine collections, the volunteers were given PABA check tablets (Laboratory for Applied Biology, London) [10] on the 24-h urine collection days. These urine samples allowed a comparative analysis of the biological effects of both conjugated (soy-texturised vegetable protein—TVP) and unconjugated isoflavones (miso).

3. Acquisition of NMR spectra and data analysis

^{1}H NMR spectra were acquired on a Bruker Avance DRX 600 spectrometer operating at 600.13 MHz at 303 K. Samples were analysed in 5-mm-od NMR tubes using a triple axis inverse (TXI) gradient probe.