A PILOT STUDY TO EXPLORE THE LINK BETWEEN HABITUAL DIET AND URINARY BIOMARKERS DURING PREGNANCY

FotiouM.[1)], KyrkouC.[1)], TsakoumakiF.[1)], DimitropoulouA.[1)], Virgiliou C.[2)], FotakisC.[3)], AthanasiadouE.[4)], Loukri A.[1)], PapadopoulosS.[1)], Stamkopoulos A.[1)], Gika H.[5)], Theodoridis G.[2)], AthanasiadisA.P.[6)],BiliaderisC. G.[1)], ZoumpoulakisP.[3)], MichaelidouA.-M.[1)]

1) Aristotle University of Thessaloniki, School of Agriculture, Department of Food Science and Technology – P.O. Box 256, 541 24, Thessaloniki, Greece – FM, fotioum@yahoo.gr; KC, ckyrkou@hotmail.gr; TF, foteinitsak@hotmail.com; DA, dimitropa@hotmail.com; LA, aploukri@agro.auth.gr; PS, sokrpapa@agro.auth.gr; SA, sasterio@agro.auth.gr; BCG, biliader@agro.auth.gr; MA-M, amichail@agro.auth.gr

2) Aristotle University of Thessaloniki, Faculty of Sciences, Department of Chemistry, 541 24 Thessaloniki, Greece – VC, cr_virgi@hotmail.com;TG,gtheodor@chem.auth.gr

3)National Hellenic Research Foundation, Institute of Biology, Medicinal Chemistry and Biotechnology – Vas. Constantinou 48, 11635, Athens, Greece – FC, bfotakis@yahoo.com; ZP, pzoump@eie.gr

4) Aristotle University of Thessaloniki, School of Medicine, 1st Department of Obstetrics and Gynecology – Papageorgiou General Hospital, Ring Road Neas Efkarpias, 56403, Thessaloniki, Greece – AE, athanasiadou.niki@gmail.com

5) Aristotle University of Thessaloniki, School of Medicine, Laboratory of Forensic Medicine and Toxicology, 541 24 Thessaloniki, Greece – GH, gkikae@auth.gr

6) Aristotle University of Thessaloniki, School of Medicine, 3rd Department of Obstetrics and Gynecology – Hippokration General Hospital, Konstantinoupoleos 49, 54642, Thessaloniki, Greece – APA, apostolos3435@gmail.com

Abstract

The aim of this pilot study was to link habitual red meat consumption and protein intake to previously established urinary biomarkers, in a group of pregnant woman. Conventional chemical analyses and metabolomics were employed to assess the maternal urine fingerprint. The % of energy derived from meat intake was correlated with urea and creatinine levels, protein intake (g) with alanine and urea concentrations, while animal protein (g) with alanine levels. Maternal pre-pregnancy BMI was associated with alanine levels. There is a link between maternal dietary intake and urinary biomarkers.

Keywords: maternal habitual diet, urine, HPLC, NMR, UPLC-MS/MS

1. INTRODUCTION

Over the last few decades, there has been a steady increase in studies that provide fundamental insights into the dynamic relationship between diet, lifestyle, genetics, and health [1].In this context, maternal nutritional status and dietary intake emerge as key factors influencing maternal and fetal immediate and long-term health [2]. Different methods – such as food-frequency questionnaires (FFQ), 24-h dietary recalls (24HDRs)and food diaries – have been employed, in order to evaluate dietary intake [3]. However, based on the multi-factorial complexities of dietary exposures, all dietary intake assessment methods are affected by both random and systematic measurement errors that influence the accuracy of dietary data [4]. For this reason,nutrition epidemiologists have utilized different biomarkers in order to evaluate nutritional status[5]. In particular, Bertram et al. (2007) ascribed the higher levels of excreted urea to the higher red meat consumption and higher animal protein intake [6], while O'Sullivan, Gibney & Brennan (2011) identified higher urinary excretion of dimethylglycine and trimethylamine N-oxide in a dietary cluster characterized by higher habitual intakes of white bread, sugars/preserves, red meat, red-meat dishes, and meat products [1].

Most of these studies are focused on general population. However, during pregnancy, the dynamic relationship between dietary intake and certain biomarkers is much more complex, as tremendous physiological forces set in motion biochemical changes, closely allied to many nutrients excretion [7].

In developed countries, meat is an important contributor to dietary protein intake [8]. Therefore, the aim of the present pilot study was to link habitual red meat consumption and protein intake to previously established urinary biomarkers in a group of pregnant women. A complementary goal was to explore whether these specific biomarkers are correlated with maternal nutrient status, as this is reflected in maternal body mass index (BMI).

2. EXPERIMENTAL

For the current pilot study, habitual red meat consumption and protein intake were assessed for 23 women in their 2nd trimester of pregnancy (18 - 22 weeks of gestation), using a validated Mediterranean oriented semi-quantitative FFQ [3]. A 24HDRof the previous day was, also, recorded. All dietary data, as well as demographic and clinical characteristics, were collected by a registered dietician.

Urine specimens were obtained under non-fasting conditions, owing to medical restrictions in controlling or restricting maternal diet during pregnancy. Urea and creatinine were determined using an automatic analyzer [9], while determination of alanine was performed by precolumn derivatization and reversed-phase high performance liquid chromatography (RP-HPLC) [10]. In this pilot study, selected urine samples were, also, analysed by applying metabolomics based methods using ultra performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) instrumentation [11] and nuclear magnetic resonance (NMR) spectroscopy [12]. Zero order and partial correlation coefficients (Pearson's *r*) were calculated in order to test the association between the study variables. Statistical analysis was performed using the SPSS version 17.0 statistical software (SPSS Inc., Chicago, IL). The significance level of all hypotheses tests was set at $p<0.05$.

The study was approved by the Bioethics Committee of the Faculty of Medicine of Aristotle University, Thessaloniki, Greece (A19479 – 26/2/08).

3. RESULTS AND DISCUSSION

The aim of this pilot study was to link habitual red meat consumption and protein intake to previously established urinary biomarkers [1, 13, 14, 15], in a group of pregnant women. The descriptive statistics concerning the study variables are given in **Table 1**.

Table 2 displays results from zero order and partial correlations between dietary variables and urine measurements. In order to illustrate the correlations between the measured variables, representative scatter plots are given in **Figure 1**. In particular, **Figure 1a** depicts the statistical significant positive correlation recorded between the percentage of energy derived from red meat consumption and creatininelevels. Total protein intake (g), as estimated by FFQ, was significantly positively correlated with urea **(Figure 1b)**. Animal protein intake (g) was associated with alanine **(Figure 1c)**.Concurrent adjustments for the effects of gestational age and maternal pre-pregnancy BMI did not alter the associations between dietary variables and urinary metabolites **(Table 2)**.

Urea is essentially a highly soluble organic waste product generated in the liver during protein catabolism and, therefore, it is considered to be an important nutritional biomarker for estimating protein intake [8, 16]. As it has already been mentioned, meat is a vital contributor to dietary protein intake and, thus, urea excretion has been associated with meat intake by numerous investigators [6, 8]. It is, also, well established that meat consumption is associated with increased excretion of creatinine [6, 8, 13, 17]. In particular, Stella et al. (2006), in an NMR-metabolomics study, reported that subjects with higher meat intake had elevated levels of creatinine in their urine specimens [13]. In addition, according to Holmes et al. (2008), urinary excretion of alanine is higher in people consuming a predominantly animal compared to a predominantly vegetable diet [18].

Table 1. Descriptive statistics of the study variables.

	Mean ± SD
% energy from red meat	6.42 ± 2.00
Total protein intake FFQ (g)	67.36 ± 9.97
Total protein intake 24HDR (g)	60.81 ± 18.33
Animal protein intake FFQ (g)	42.63 ± 8.10
Animal protein intake 24HDR (g)	37.56 ± 15.57
pre-pregnancy BMI (kg/m^2)	24.02 ± 5.01
Urea (mg/dL) *	870.35 ± 482.79
Creatinine (mg/dL) *	82.68 ± 49.96
Alanine (μM) *	287.34 ± 169.85

* The results presented are obtained by applying conventional analyses.

Table 2. Zero order and partial correlations between dietary variables and urine measurements.

		Zero Order Correlations			Partial Correlations[1]			
		Alanine (μM)	Urea (mg/dL)	Creatinine (mg/dL)		Alanine (μM)	Urea (mg/dL)	Creatinine (mg/dL)
% energy from red meat	*r*		0.48	0.50	*r*		0.50	0.54
	p		0.02	0.01	*p*		0.02	0.01
Total protein intake FFQ (g)	*r*	0.43	0.52		*r*	0.42	0.49	
	p	0.04	0.01		*p*	0.05	0.02	
Total protein intake 24HDR (g)	*r*	0.50	0.49		*r*	0.43	0.47	
	p	0.02	0.02		*p*	0.05	0.03	
Animal protein intake FFQ (g)	*r*	0.47			*r*	0.54		
	p	0.02			*p*	0.01		
Animal protein intake 24HDR (g)	*r*	0.50			*r*	0.43		
	p	0.01			*p*	0.05		
pre-pregnancy BMI (kg/m^2)	*r*	0.54			*r*	0.56[2]		
	p	0.01			*p*	0.01		

[1]Controlling for gestational week & pre-pregnancy BMI
[2] Controlling only for gestational week

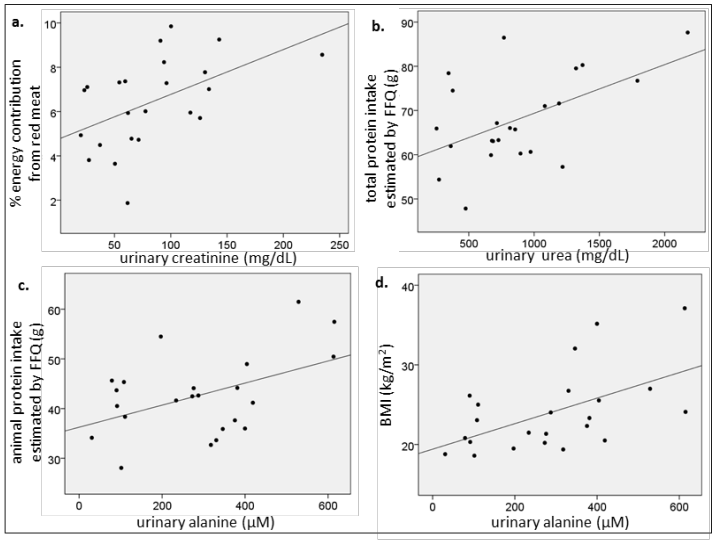

Figure 1.Representative scatter plots between a. energy derived from red meat intake and urinary creatinine, b. total protein intake and urinary urea, c. animal protein intake and urinary alanine, and d. maternal pre-pregnancy BMI and urinary alanine.

Concerning the second goal of the pilot study, maternal pre-pregnancy BMI was positively correlated with alanine levels in urine **(Table 2 - Figure 1d)**. Several studies have indicated clear BMI-related metabolic changes in amino acids excretion, as obese subjects tend to show an increase in protein turnover [19]. In particular, Holmes et al. (2008), in an NMR-based metabonomics approach, found that BMI and urinary alanine were positively correlated [18]. At this point, it should be emphasized that this particular amino acid may play a crucial role in fetal growth and development [20].

The findings of the present study unveiled significant associations between the habitual dietary intakes of pregnant women and certain nutritional biomarkers in urine. Considering the potential importance of gene-diet/nutrient interactions, a more holistic approach to dietary biomarker identification may be very informative

[4]. The inclusion of different metabolomics technologies - such as NMR and HILIC-MS/MS - is likely to be fruitful, in order to identify metabolic variations during pregnancy with respect to various maternal dietary regimes, patterns or interventions.

ACKNOWLEDGMENTS

This research has been co-financed by the European Union (European Social Fund—ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)—Research Funding Program: Thales. Investing in knowledge society through the European Social Fund.

REFERENCES

[1] A. O' Sullivan, M.J. Gibney, L. Brennan, "Dietary intake patterns are reflected in metabolomic profiles: potential role in dietary assessment studies". Am. J. Clin. Nutr., 93(2) (2011) pp. 314-321.

[2] D.P. Barker, "Developmental origins of chronic disease". Public health, 126(3) (2012) pp. 185-189.

[3] E. Athanasiadou, C. Kyrkou, M. Fotiou, et al., "Development and validation of a Mediterranean oriented culture-specific semi-quantitative food frequency questionnaire". Nutrients, 8(9):522 (2016) pp. 1-20.

[4] M. Jenab, N. Slimani, M. Bictash, P. Ferrari, S.A. Bingham, "Biomarkers in nutritional epidemiology: applications, needs and new horizons". Hum. Genet., 125(5-6) (2009) pp. 507-525.

[5] J. Cade, R. Thompson, V. Burley, D. Warm, "Development validation and utilization of food-frequency questionnaires—a review". Public Health Nutr., 5 (2002) pp. 567–587.

[6] H.C. Bertram, C. Hoppe, B.O. Petersen, et al., "An NMR-based metabonomic investigation on effects of milk and meat protein diets given to 8-year-old boys". Br. J. Nutr., 97(04) (2007) pp. 758-763.

[7] S. Miller, V. Ruttinger, I.G. Macy, "Urinary excretion of ten amino acids by women during the reproductive cycle". J Biol Chem, 209 (1954) pp. 795-801.

[8] L.O. Dragsted, "Biomarkers of meat intake and the application of nutrigenomics". Meat sci, 84(2) (2010) pp. 301-307.

[9] M. Fotiou, A.M. Michaelidou, A.P. Athanasiadis, et al., "Second trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal prepregnancy BMI and birth weight centiles". J. Matern. Fetal Neonatal. Med., 28(8) (2015) pp. 910-915.

[10] A.P. Athanasiadis, A.M. Michaelidou, M. Fotiou, et al., "Correlation of 2nd trimester amniotic fluid amino acid profile with gestational age and estimated fetal weight". J. Matern. Fetal Neonatal. Med., 24(8) (2011) pp. 1033-1038.

[11] C. Virgiliou, I. Sampsonidis, H.G. Gika, N. Raikos, G.A. Theodoridis, "Development and validation of a HILIC-MS/MS multitargeted method for metabolomics applications". Electrophoresis, 36.18 (2015) pp. 2215-2225.

[12] C. Fotakis, M. Zoga, C. Baskakis, et al., "Investigating the metabolic fingerprint of term infants with normal and increased fetal growth". RSC Adv, 6(83) (2016) pp. 79325-79334.

[13] C. Stella, B. Beckwith-Hall, O. Cloarec, et al., "Susceptibility of human metabolic phenotypes to dietary modulation". J. Proteome Res., 5(10) (2006) pp. 2780-2788.

[14] M. Beckmann, A.J. Lloyd, S. Haldar, et al., "Dietary exposure biomarker-lead discovery based on metabolomics analysis of urine samples". P. Nutr. Soc., 72(03) (2013) pp. 352-361.

[15] L.G. Rasmussen, H. Winning, F. Savorani, et al., "Assessment of the effect of high or low protein diet on the human urine metabolome as measured by NMR". Nutrients, 4(2) (2012) pp. 112-131.

[16] S.S. Heinzmann, I.J. Brown, Q. Chan, et al., "Metabolic profiling strategy for discovery of nutritional biomarkers: proline betaine as a marker of citrus consumption". Am. J. Clin. Nutr., 92.2 (2010) pp. 436-443.

[17] A.J. Cross, M.M. Jacqueline, S. Rashmi, "Urinary biomarkers of meat consumption". Cancer Epidemiol. Biomarkers, 20.6 (2011) pp. 1107-1111.

[18] E. Holmes, R.L. Loo, J. Stamler, et al., "Human metabolic phenotype diversity and its association with diet and blood pressure". Nature, 453(7193) (2008) pp. 396-400.

[19] L. Brennan, "Session 2: Personalised nutrition Metabolomic applications in nutritional research: Symposium on 'The challenge of translating nutrition research into public health nutrition'". Proc. Nutr. Soc., 67.4 (2008) pp. 404-408.

[20] G. Wu, "Amino acids: metabolism, functions, and nutrition". Amino acids, 37.1 (2009) pp. 1-17.