An analytical method to investigate the metabolites and co-factors of the methionine-homocysteine cycle in women using combined oral contraceptives and non-users

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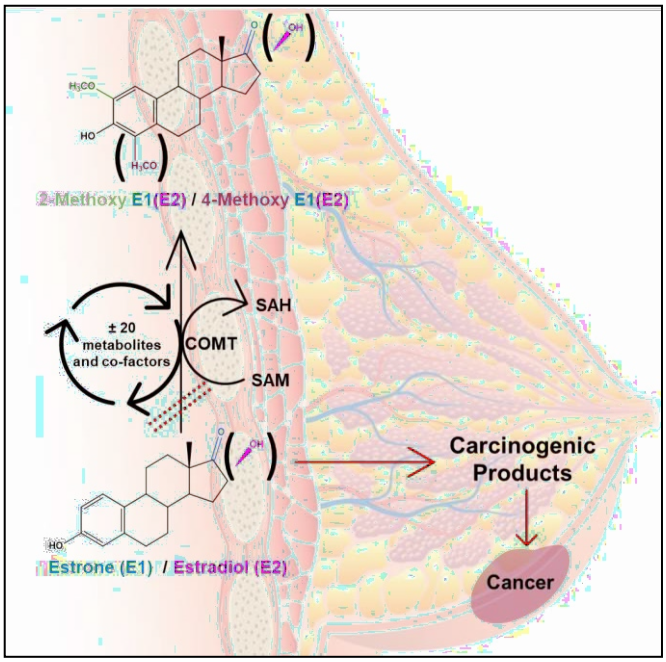

Figure 1: The ineffective methylation of estrogens can lead to an increase in the formation of carcinogenic products and possibly cancer (red lines and arrows). The effective activity of the enzyme COMT and metabolite SAM (produced in the methionine-homocysteine cycle) is important for effective biotransformation of estrogens (black arrows).

The process of biotransformation protects the body from endogenous and exogenous substances like combined oral contraceptives (COCs). During biotransformation, estrogen undergoes methylation with the assistance of catechol-O-methyltransferase (COMT) to limit the production of carcinogenic products. Effective COMT activity is crucial to limit the formation of these carcinogenic products. The importance of estrogen methylation is demonstrated by the fact that women with polymorphisms in the COMT-pathway, which negatively affect the rate of methylation, are more prone to develop breast cancer. The metabolite S-adenosyl methionine (SAM) produced in the methionine-homocysteine cycle, acts as the methyl donor during methylation of catechol estrogens. At the Biotransformation and Oxidative Stress Status Laboratory (BOSS lab) of the NWU, the urinary hormone metabolite profile of clinically referred patients is routinely analysed. Problems with the methylation metabolism is reflected by low levels of the methoxy-estrogens and increased levels of the glutathione conjugates and DNA adducts. After some of these patients have been treated with a co-factor cocktail that promote the detoxification of the catechol estrogens and the quinones, their profiles changed showing improved methylation and less adduct formation. Themethionine-homocysteine cycle is a multi-step cycle including a great number of metabolites and co-factors. In order to determine more precisely how the methionine-homocysteine cycle influences the methylation of estrogens and how it is influenced by the use of COCs (and other xenobiotics), a targeted analytical method is required to successfully quantify the metabolites and co-factors of the methionine-homocysteine cycle. The aim of this study was to develop a targeted metabolomics method which could detect most of the important intermediates in the methylation cycle. The method used by Guiraud *et al* (2017) was implemented. This method is now being optimised to quantify metabolites and co-factors of the methionine-homocysteine cycle in serum samples, using chromatographic separation with a trifunctional C18 column together with an ion-pairing agent. Tandem mass spectrometry is used for highly selective detection. We present here the initial validation results of this method anddemonstrate the application thereof on selected clinical cases.

References:

Guiraud, S.P., Montoliu, I., Da Silva, L., Dayon, L., Galindo, A.N., Corthésy, J., ... Martin, F.-P. 2017. High-throughput and simultaneous quantitative analysis of homocysteine–methionine cycle metabolites and co-factors in blood plasma and cerebrospinal fluid by isotope dilution LC–MS/MS. Analytical and bioanalytical chemistry, 409(1):295-305. doi:10.1007/s00216-016-0003-1

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