# Importance of Metabolomics Technology in Advanced Physiology

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The complex biological process of reproduction results in changes in energetics and physiological function (Speakman, 2008). Accumulated research data conducted on the energetic changes during reproduction, particularly for mammals in - lactation, have shown that energy intake and total energy expenditure are enormously increased ((Johnson et al., 2001; Speakman, 2008). However, very little is known about the levels of metabolites associated with differences in energy metabolism during lactation. Metabolomics is defined as the identification of all small metabolites (<1000 Da) at a cellular level of any given tissue (the metabolome). These metabolites are comprised of primary compounds, intermediate compounds in metabolic pathways and the end products of cellular processes (Boccard et al., 2010; Nicholson et al., 1999).

The first use of metabolomics in the analysis of cellular processes was to determine hundreds of molecules related to redox- state in rat mitochondria (Kristal et al., 1998). Since that time, considerable attention has been given to this area and hundreds of papers were later published about metabolomics (reviewed in Kell, 2004) Metabolomics is commonly used to monitor progression of a disease and the response of therapeutic interventions (Gates & Sweeley, 1978) with a view to potentially identifying early biomarkers of disease or identification of markers of disease progression. However, one of the most important applications of using metabolomics technology is in systems biology (Jewett et al., 2006; Wang et al., 2006). Systems biology is the quantitative study of the whole organism by looking at it as a web of the interactions between molecular components such as DNA, mRNA, proteins, and

metabolites with its environment (Nielsen & Jewett, 2007). Constructing systems biology networks is therefore considered to be a novel tool to investigate phenotypic changes and the interaction between phenotype and genotype (Allen et al., 2003; Raamsdonk et al., 2009; Nicholson & Wilson, 2003). Metabolome analysis can be achieved by two different approaches. Firstly, the metabolite profile method (non-targeted metabolomics), in which a large number of metabolites (> 1000 metabolites) are detected. When these are comprised of intracellular metabolites that are commonly known as a metabolomic fingerprint (endometabolome) and extracellular metabolites being called a metabolomic footprint (exometabolome) ((Goodacre et al., , 2004; Kell, 2004). The second approach used in metabolomics is a targeted method, through which a relatively small number (<10 metabolites) of specific metabolites can be absolutely measured. However, the number of metabolites detected by the target method has been recently expanded due to advancements in technology (Nielsen & Jewett, 2007; Soltow et al., 2010; Villas-Bôas et al., 2005).

Although the majority of small metabolite quantification is performed by applying separation techniques such as liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis (CE) coupled with mass spectrometry (MS) or Nuclear magnetic resonance (NMR), it is practically impossible to detect all small molecules in a biological tissue at a given state due to the limitations in the sample extraction method, chemical variety, and the dynamic range of the metabolome (Goodacre et al., 2004). Nevertheless, a considerable advancement has been made regarding metabolites identification, with around 7,000 human metabolites identified by 2009 compared to 2180 metabolites known 2 years previously (Wishart et al., 2009). Currently, several thousand metabolites can be identified, depending on the analytical technology available (Soltow et al., 2010).

Although there is no unique approach that can identify the whole metabolome, non-targeted metabolomics can generally be measured using both MS and NMR (Adamski & Suhre, 2013). and can be used when the goal is to discover novel pathways among samples, such as diseased versus healthy samples or mutant versus wild samples (Adamski and Suhre, 2013). Although both genomics and proteomics have extensively been used to investigate how DNA is accurately reproduced and how proteins function and are structured, studying metabolomics can give further advantages (Soltow et al., 2010). For example, previous data of experimental method of metabolic analysis has indicated that alterations in the enzymatic activities at the level of the individual have considerable effects on the levels of the variety of individual's metabolites. However, very few effects were observed in the chemical changes and molecular mechanisms associated with the genome (Raamsdonk et al., 2001). In addition, changes in metabolites are thought to be amplified relative to changes observed at the genomic and proteomic levels. This is attributed to the fact that the metabolomes lie further along the road from gene to function, which in turn could provide a clearer picture of cellular activity at a functional level (Soltow et al., 2010).

Understanding the life –history traits (and ageing) is growing by using a metabolomics technology that measures the metabolic profile linked to the ageing process (Soltow et al., 2010). Lawton et al. (Lawton et al., 2008) studied the changes in metabolome of 269 human plasma samples in relation to age and demonstrated that more than one hundred metabolites associated with protein, lipids, and oxidative stress metabolism were positively correlated with ageing. In a similar study, more than 131 metabolites identified from 2,162 serum samples of healthy human individuals with an age range from 32-81 years were observed to be positively related

with age(Yu et al., 2012) Most of these identified metabolites were closely linked to dysfunction of mitochondrial fatty acid beta-oxidation (Yu et al., 2012). In aged rats, metabolomics measurements of urine also indicated that many of the intermediate metabolites levels in the tricarboxylic acid were downregulated combined with increased oxidation of antioxidants (Schnackenberg et al., 2007). This result is consistent with idea that ageing is linked with lowered mitochondrial function and elevation of reactive oxygen species (ROS).

Although the traditional concept that reproduction should lead to a reduction in later reproductive performance of an animal and/or reduced longevity, few studies have examined the metabolic profile during lactation. For example, results from studying 44 metabolites in milk of dairy cows at early lactation have shown that markers related with ketosis such as acetone and β-hydroxybutyrate were increased (Klein et al., 2010). Analysis of the plasma metabolic profiles of lactating sows showed that towards the end of lactation, significant changes in their lipid metabolites could be observed, compared to these metabolites detected before parturition (Hedemann et al., 2012). It has recently been that the trade-off between lactation and oxidative damage is probably not as simple as previously believed due to the lack of a generic oxidative damage marker accommodating all types of oxidative damage and also the damage to any given tissue can also happen in any potential target within a cell. Therefore, the analysis of a whole metabolomic profile during lactation could provide new insights in relation to the oxidative stress theory by expanding the scope of the oxidative stress markers.

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