## Summary

Nutrients and bioactive components present in fruits and vegetables interact with several metabolic pathways and physiological functions leading to a potential reduction of the risk of diseases. Metabolomics is a methodology that allows the measurements of hundreds of compounds in biological samples at once, and therefore, it allows exploring the effect of food in a more global way compared to traditional techniques.

The aim of this PhD project was to uncover the effect of consumption of apple or apple products on biochemical mechanisms and explore health related associations by using an LC-MS based metabolomics approach. To accomplish this aim both rat and human models were investigated and later compared the effects and usefulness of different samples for profiling.

The first study was an explorative approach to investigate the effects on the plasma metabolome after 80 male rats were fed with a diet supplemented with two dosages (5 g/day and 10g/day) of whole fresh apple pieces through 16 weeks (PAPER I). Plasma samples were analysed by LC-MS. Due to the complexity of the design of the study, several chemometric analyses were employed. Initially, ASCA was applied in order to isolate the metabolic variations related to the consumption of fresh apples, successively PLS-DA to reveal discriminative metabolites in control vs. apple fed rats and finally PLS analysis to investigate possible dose-responses. Findings are related to a decrease in toxic catabolic products produced by protein fermentation in the gut and to an increase of a protective metabolite after apple intake. Furthermore, apple consumption seems to reduce some carboxylic acids, amides, long- and medium-chain acyl-carnitine species as well as some long chain lysoPCs, corticosterone and mineralocorticoids, and few other compounds.

The rat study was followed up on a randomised, controlled 5x4 weeks human cross-over study, introducing apple products (clear and cloudy juices, and apple pomace) and plasma metabolic profiles were investigated (PAPER II). PLS-DA analysis was employed in a pairwise fashion (control period vs. each of the other periods) in order to select the important features discriminating the difference between control and apple or apple products supplementations. Whole apples and pomace were characterised by having the strongest effect to decrease the levels of branched-chain amino acids and their gut microbial fermentation products as well as short and medium chain acyl-carnitines. Furthermore, lower levels of primary and secondary bile acids and some lysophospholipids were observed with apple and apple product supplementations, while levels of uric acid increased.

In PAPER III, 24 h urine metabolic profilings from the same human study presented in PAPER II were investigated. The research is still under progress and the initial investigation was targeted to the effects of apple and clear juice supplementations by employing *i*ECVA. Preliminary results show again a more prominent effect from apple than apple juice with possible exposure markers, including phase 2 metabolites of flavonoid degradation products and chalcones. Furthermore, in urine

data, a larger number of markers increased after the apple and juice intervention - compared with control - respect to plasma.

Overall, the studies included in this project showed that fresh apple (and pomace) potentially promotes several health effects by decreasing toxic metabolites, amino acids catabolism, bile acids reabsorption, and lipid catabolism. Moreover, it seems that apple acts consistently in both rats and humans, although the revealed metabolites partially differ.

## List of publications

**PAPER I**

**Rago, D.**, Mette, K., Gürdeniz, G., Marini, F., Poulsen, M., Dragsted, L. (2013) A LC–MS metabolomics approach to investigate the effect of raw apple intake in the rat plasma metabolome. *Metabolomics*, 9, (6): 1202-1215.

**PAPER II**

**Rago, D.**, Gürdeniz, G., Ravn-Haren, G., Dragsted, L. (2013) The effect of apple and apple products on the human plasma metabolome investigated by LC-MS profiling. Submitted to *Metabolomics*.

**PAPER III**

**Rago, D.**, Gürdeniz, G., Ravn-Haren, G., Dragsted, L. (2013) The effect of apple and apple products on the human urine metabolome investigated by LC-MS profiling. Draft paper

## Supplemental Material

Gürdeniz, G., **Rago, D.**, Bendsen, N. T., Savorani, F., Astrup, A., Dragsted, L. O. (2013) Effect of trans-Fatty Acid Intake on LC-MS and NMR Plasma Profiles. *PLoS One, 8*(7).

E. Acar, G. Gurdeniz, M. Rasmussen, **D. Rago**, L. Dragsted, R. Bro (2012) Coupled Matrix Factorization with Sparse Factors to Identify Potential Biomarkers in Metabolomics, *International Journal of Knowledge Discovery in Bioinformatics*, 3: 22-43.

# TABLE OF CONTENTS