

Flavanol rich-foods such as cocoa can modify urine metabolomics both in humans and rats in healthy conditions. However, the metabolic responses of cocoa supplementation in diabetes remains to be considered. Therefore, in the present study, we applied untargeted metabolomics to identify potential biomarkers of altered metabolic pathways in Zucker Diabetic Fatty (ZDF) rats and their potential association with the improvement of metabolic variables in diabetic animals supplemented with cocoa. Male ZDF rats were fed on standard (ZDF-C) or 10% cocoa-rich diet (ZDF-Co) from week 10 to 20 of life. At the end of the study, glucose tolerance test (GTT), glycemia, insulinemia, HbA1c, HDL cholesterol, LDL cholesterol and triglycerides were analyzed. Insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR). Urine samples (23-h) were collected and analyzed by an untargeted <sup>1</sup>H NMR spectroscopy-based metabolomic approach. We visualized the differences between samples with Principal Component Analysis. The analysis of its loadings of the two first principal components was used to identify the most relevant metabolomic variables. Relationship strength between parameters was assessed using the two tailed Pearson's correlation test. Our results showed that long-term treatment with cocoa clearly decreased serum glucose levels and improved glucose metabolism in ZDF rats. Likewise, metabolomics analysis revealed that cocoa intake significantly reduced the urine levels of glucose, urea and 4-hydroxyphenyllactate and increased those of glycine and hippurate in diabetic animals. Interestingly, there were also differences univocally associated with cocoa supplementation such as increased levels of 2-oxo-glutarate and branched amino-acids (valine, leucine, isoleucine) and decreased levels of acetoacetate and 3-indolesulfate<sup>1</sup>. This study demonstrates that cocoa supplementation significantly affects urine metabolomics in ZDF rats and may provide new insights into the metabolic routes by which cocoa flavanols exert their beneficial effects on diabetes.