

## Metabolomic approach to screen sex differences in clinical biochemistry

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Metabolomic profiling has been proven to be a good tool to explore cellular variations at the molecular level. Notably, serum metabolite level is essential to have a direct readout of biological response. Associations between sex and gender with the incidence, prevalence, signs and symptoms, and severity of a disease, as well as the efficacy of drug treatments are well known. However, the most of studies did not consider sex and gender; therefore results are not stratified for these variables (Kim et al. 2010). The present study individuates sex-specific differences of serum amino acids and free and esterified carnitine levels in healthy young donors of both genders being the women also stratified for oral contraceptive (OC) use. Healthy adult men and women with regular menstrual cycles (28 days) aged between 18 and 40 years were enrolled; the subjects were free of diseases or infections disease for at least two months prior to the study and none of them were on long-term medications, with the exception of OC for women. All women were analysed during the follicular phase (1–10 days) and OC all belonged to the third generation of combined contraceptives (estrogen + progestin). Aminoacids and Acylcarnitine were analysed by HPLC or LCMSMS according to published protocols (Carducci et al., 1996; Catanzano F, et al., 2010)

The serum level differences between men and females of free and esterified carnitine coincide with previous findings showing that carnitine and esterified carnitines are higher in males than in females (Slupsky, et al. 2007; Reuter et al 2008). Analysing the single esterefied carnitine emerges that the differences are significant for C5, C12, C16, C18, C5:1, C10:2, C14:2, C16:1 and C18:2. The OC use reduces either free carnitine or esterified carnitine suggesting that serum carnitine is regulated by sexual hormones. Examining single esterefied carnitines emerges that the variations are significant for C6:1, C8:1, C10:2, C18:1. The following amino acids: glycine, alanine, methionine, serine, arginine, hydroxyproline, ornitine, and citrulline do not diverge between male and female; while proline, valine, leucine, isoleucine, phenylalanine, tyrosine, asparagine, glutamine+glutamate, aspartate, glutamate, hystidine and taurine are higher in men than in women. Thryptofan and cysteine are lower in men than in women. The OC use significantly decreases the level of glycine, alanine, proline, methionine, tyrosine, hydroxyproline, ornitine, citrulline and taurine, whereas it does not significantly change the others. Notably, the decrease in methionine could be linked to the decrease observed in carnitines because the endogenous synthesis of carnitine starts from L-lysine and L-methionine (Marcovina et al, 2013). The variations in free carnitine and esterified carnitines and in amino acids levels are also associated with differences in ematological and routinary biochemical biomarkers. In conclusion, we evidence that the serum metabolites depict a sexual dimorphism in the most of the case. Notably the use of OC modifies the metabolite profile in women. The results of this study also suggest that a gender approach should be adopted in design and interpretation of studies.

### References

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