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**Title:** Study protocol: a 2-month cross-over controlled feeding trial investigating the effect of animal and plant protein intake on the metabolome and cardiometabolic health

**Objective:** A dietary shift in favor of plant protein (PP) sources over animal protein (AP) sources has been advocated for both sustainability and health reasons, this dietary transition being noticeably associated to decreased cardiovascular and diabetes risks. The differences in amino acid composition between PP and AP may have several effects on the metabolic pathways, and in turn health impacts, which are still poorly characterized. This project aims at characterizing, with a combination of “omics” approaches, the metabolic reorientations induced by a dietary shift from AP to PP sources and understanding their health effects in a population at cardiometabolic risk.

**Methods:** We will conduct a cross-over randomized feeding trial ([NCT04236518](https://clinicaltrials.gov/ct2/show/study/NCT04236518)) in 20 healthy overweight males (BMI 25-35), aged 25-55, with an enlarged waist circumference (> 94cm) and high plasma triglycerides (>1.5g/L). Participants will be assigned for 1 month each to 2 diets containing predominantly either AP (65% AP:35% PP) or PP (35% AP:65% PP) in a randomized order, separated by a 2-week wash-out period. Lunch and diner will be directly provided while dietary guidelines will be given for breakfast and snacks. Blood, urine and stool samples will be collected at the fasted state every 2 weeks. At the end of each dietary intervention, blood and urine will be collected following a high fat meal, which challenges metabolism and vascular homeostasis. Plasma and urine non-targeted metabolomics analyses (LC-MS) will be combined with Peripheral Blood Mononuclear Cell (PBMC) transcriptomics and fluxomics analyses (D<sub>2</sub>O tracer) to get a comprehensive overview of the metabolic phenotype associated with AP or PP intake. Flow-Mediated Dilatation (FMD) and Flow Laser Doppler (FLD) will be used to measure respectively macrovascular endothelial function and microvascular skin blood flow at the fasted state and after the high-fat meal. We will also measure anthropometric parameters and analyze biochemistry and inflammatory markers.

**Results:** We expect the multi-omics fingerprinting to reveal subtle metabolic differences associated to AP or PP intake, with a positive effect of PP intake. Improved inflammatory status and endothelial function are also expected to be associated to PP intake.

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