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Gaïa Lépine, François Mariotti, Jean-François Huneau, Nathalie Poupin, Marie Tremblay-Franco, et al.. 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. Nutrition Live Online 2021, Jun 2021, Online, United States. 5 (Supplement_2), pp.1281, 2021, 10.1093/cdn/nzab057_011 . hal-03292189

HAL Id: hal-03292189

<https://hal.science/hal-03292189v1>

Submitted on 6 Dec 2021

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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

Abstract ID: P28-011-21



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Context:

- A dietary shift from **animal protein (AP)** to **plant protein (PP)** advocated for sustainability and health reasons (lower cardiovascular and diabetes risk)
- Mechanistic bases underlying these associations still poorly characterized and involving numerous metabolic pathways.

Objective:

This protocol aims at characterizing the metabolic reorientations induced by AP → PP dietary shift and understanding their health effects in a population at cardiometabolic risk, combining a highly controlled feeding trial and “omics” analytical approaches (NCT04236518).

1. A population at cardiometabolic risk

This ongoing study targets the **hyper-triglyceridemic enlarged waist circumference phenotype**

Inclusion criteria

- Men
- Age: 25 to 55 years
- BMI: 25-35 kg/m²
- Waist circumference > 94cm
- Plasma triglycerides > 1,5g/L

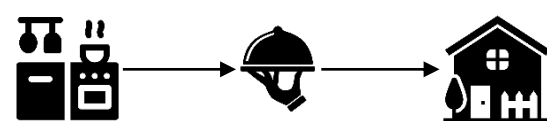
Recruitment objective: n=20 subjects



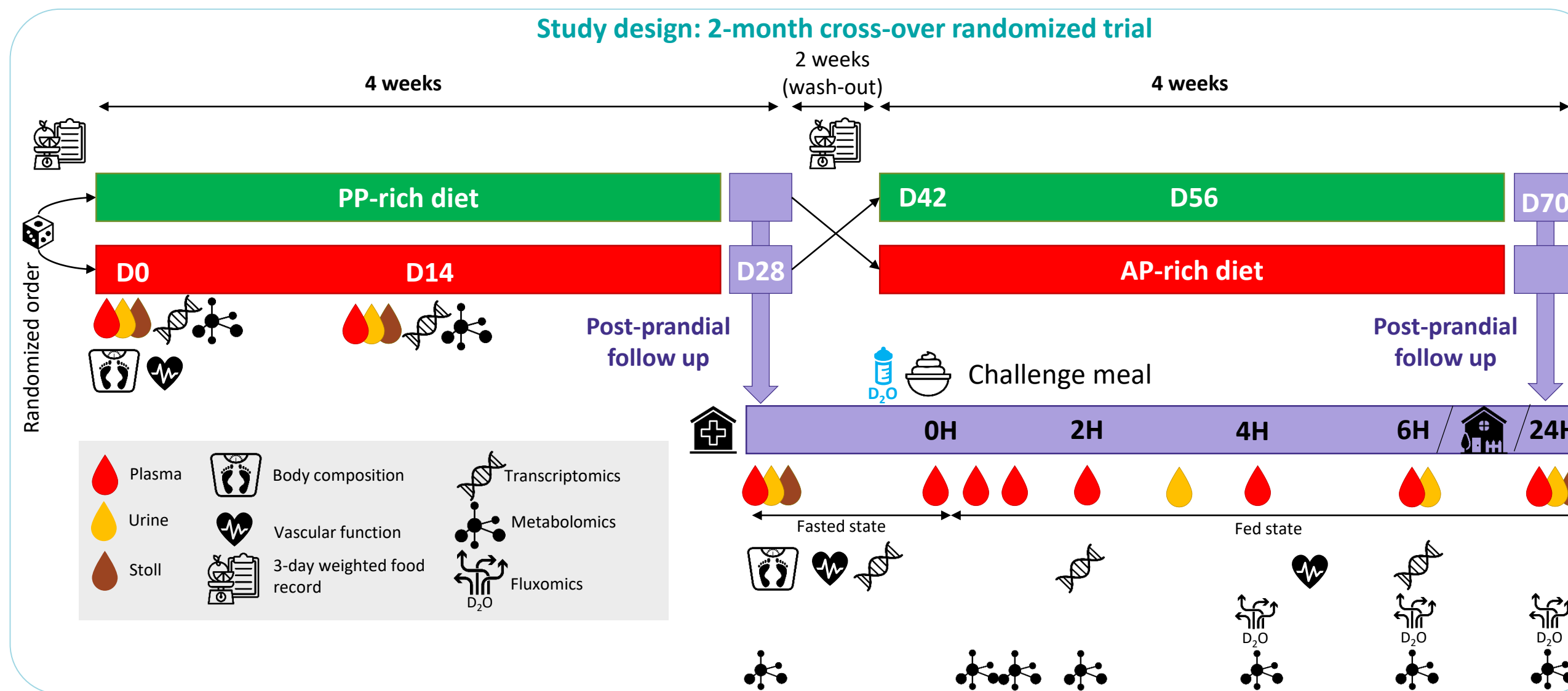
	Mean (SD)
n	6
Age (y)	44.2 (8.0)
BMI (kg/m ²)	29.4 (2.8)
Waist circumference (cm)	102.8 (5.5)
Plasma triglycerides (g/L)	1.96 (0.57)

Table 1: Description of the sample already included

2. A control feeding trial in a free living environment



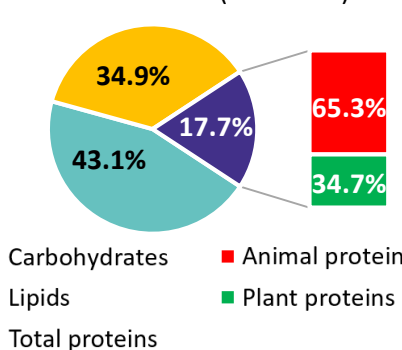
- Ready-to-eat lunches and dinners freshly prepared by a catering service
- Dietary advices regarding breakfast and snacks
- Compliance follow-up with dietary dairies and blood measurement of natural abundance of nitrogen isotope (AP and PP have distinct natural isotopic signatures)
- 3-day weighted food records (before intervention and during wash-out) for characterization of habitual food intake



3. AP-rich and PP-rich diets

- AP-rich diet ↔ western omnivore diet (65% AP:35% PV)
- PP-rich diet ↔ flexitarian diet including small amounts of meat and dairy (36% AP:64% PV)
- Both diets mixed all the major animal and plant sources, without any exclusion

AP-rich diet (2106 kcal)



PP-rich diet (2017 kcal)

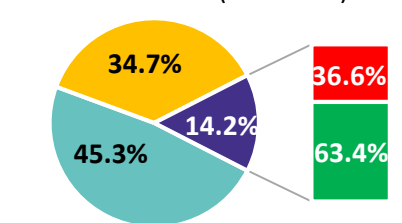


Figure 1: Mean daily macronutrients contribution to total energy intake provided by ready-to-eat meals

Intake (g/d)	PP-rich diet	AP-rich diet
Red and processed meat	10.0	166.4
Dairy	115.7	158.6
Fish	22.9	58.6
White meat	40.0	98.6
Eggs	41.4	35.7
Legumes	225.7	64.3
Fruits and vegetables	638.6	580.0
Grains and potatoes	432.9	461.4
SFA	31.5	30.4
Fiber	37.7	29.2
Sugar	72.1	70.7

Table 2: Mean dietary composition of ready-to-eat meals provided in AP and PP-rich diets

4. Assessing metabolic flexibility following a high-fat meal

Postprandial follow-up after a high-fat challenge (identical in each cross-over arm) allows to measure **adaptation capacity under challenging postprandial conditions** (high influx of saturated fatty acids and sugar) and is expected to reveal subtle differences induced by the dietary intervention which could not be visible at the fasted state, when tight homeostatic processes still prevail.



Challenge meal composition:

900kcal (80% lipid, 13% carbohydrate, 7% protein)
 Cream 30% fat (267g) + white sugar (20g) + whole milk protein isolate (11,5g)

What is new about this study?

Very little is known regarding the metabolomics signature associated to AP and PP intakes in humans (see poster P11-019-21) and up to day, no RCT has specifically investigated the effects of the AP → PP dietary shift using a combination of “omics” approaches. **This study will bring new hypotheses regarding the metabolic pathways affected by AP and PP-rich diets and associated biomarkers and will characterize with high-end methods the effects on endothelial function.**

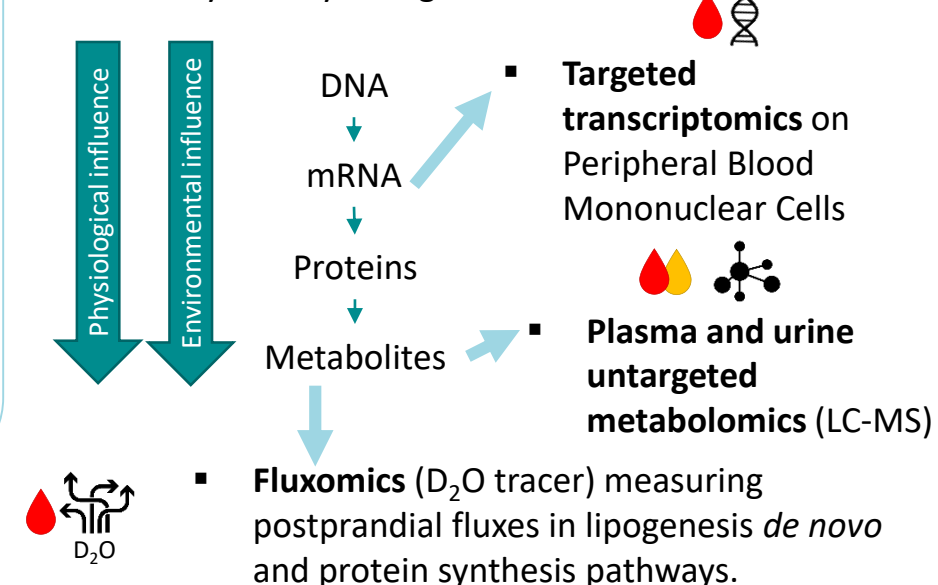
Effects on cardiometabolic health

5. Overall health outcomes

- Body composition measured by impedancemetry
- Glucose homeostasis, lipid and protein metabolism assessed by clinical biochemistry
- Inflammation status (cytokines)

6. Multi-omics phenotyping

The combination of omics approaches, providing a **multilevel phenotyping**, will give the possibility to characterize very subtle metabolic reorientations induced by dietary changes.



7. Measurement of endothelial function

- Circulating markers of endothelial function: plasma nitrite and nitrosothiols
- Flow Laser Doppler (FLD): measurement of hand microcirculation
- Flow Mediated Dilatation (FMD): measurement of brachial artery dilatation induced by blood flow changes

