Study design

**Cohort description:**
- \( N_{\text{initial}} = 102 \)
- \( N_{\text{final}} = 77 \) individuals

HIV+ during ART regimen. No antibiotics consumption 3 months before the study initiation. CD4 counts > 500 cells/mL.

**Intervention strategy:**
- 50 g/day Extra Virgin Olive Oil
- 30g/day walnuts

**Follow up:** 3 months (12 weeks)

**Groups:**
- Habitual diet -> **Control** group
- Habitual diet+ **supplemented with extra Virgin Olive Oil and walnuts** -> **Diet** group

The PREDIMED Trial in HIV individuals

Following the PREDIMED guidelines.

**Strategies to identify the individuals adhesion to the established diet:**
- **Interview** with specialized dietitian to improve their diet style.
- Tests:
  - **Adherence**: scores 1-14.
    - High adherence: > 10 points
    - Good adherence: 7-10 points
    - Low Adherence: < 7 points
  - **Ingested** aliments: list of more than 150 aliments.
  - **Dietetic register**: individuals had to register all aliments ingested in the 7 days before to the study initiation.
- **Fulfilment markers**:
  - In blood: alpha linoleic acid.
  - In urine: olive oil-derivate metabolites.

**Time-line:**
- Week 0
- Week 12
Methods

Towards standards for human fecal sample processing in metagenomic studies.
Nat Biotechnol. 2017

DNA extraction method

- QIAGEN DNA stool minikit
- Zircònias beads 0.1mm
- Bead shaker
- Without Inhibitex tablet
- Amonium acetate 10M, isopropanol and EtOH 70%

Bioinformatic analysis: QIIME 2 software

RAW sequences

- Quality control
- “Trimming” and filtering
- Union of Forward and Reverse
- OTU assignment
- “Chimeras” removal
- Taxonómica assignment
- Statistical analysis

In collaboration with:

CRG Centre for Genomic Regulation

BSC Barcelona Supercomputing Center
Centro Nacional de Supercomputación
Results

The PREDIMED Trial in HIV individuals

**MICROBIOME**

**α & β diversity**

**By Intervention group**

- **Control group**: Habitual diet
- **Diet group**: Habitual diet + supplemented with extra Virgin Olive Oil and walnuts

Principal Coordinates Analysis (PCoA):

By Intervention group

Phyllum diversity

Evennes

NO differences between intervention groups in Alpha diversity
Results

The PREDIMED Trial in HIV individuals

**MICROBIOME**

α & β diversity

Principal Coordinates Analysis (PCoA):

By Adherence punctuation

Adherence: scores 1-14:
- High adherence: > 10 points
- Good adherence: 7-10 points
- Low Adherence: < 7 points

GeSIDA 2019

Individuals who performed the intervention with **High Adherence Score** presented more:
- **OTU counts** and **Phylum Diversity**

Observed OTUs – Total counts

Phylum diversity

Kruskal-Wallis, p = 0.0089

0.061
0.37

0.12

Low
Normal
High

Faith PD (Phylum Diversity)
Results

The PREDIMED Trial in HIV individuals

MICROBIOME

**Enriched bacterial genus in High and Low Adherence to EVVO**

**Low Adherence:**
Bacteroides, Parabacteroides, Desulfovibrio, Paraprevotella, Bilophila.

**Hihg Adherence:**
Burkholderiales, Butyvibrio, Catenibacterium, Succinivibrio

LEfSe analysis

LDA SCORE (log 10)
**Results**

**MICROBIOME**

The PREDIMED Trial in HIV individuals

By Adherence punctuation

Relative abundances

Enriched bacterial genus in High and Low Adherence to EVVO

- **High-Adherence**
- **Low-Adherence**

**Low Adherence:**
- Bacteroides
- Parabacteroides
- Desulfovibrio
- Paraprevotella
- Bilophila

**High Adherence:**
- Burkholderiales
- Butyvibrio
- Catenibacterium
- Succinivibrio

**LEfSe analysis**

- Bacteroides
- Streptococcus
- Desulfovibrio
- Sutterella
- Desulfovibrionales
- Parabacteroides
- Deltaoteobacteria
- Rikenellaceae
- Alistipes
- Barnesiellaceae
- Paraprevotella
- Bilophila
- Barnesiella
- Succinivibrionaceae
- Clostridium
- Coriobacteriaceae
- Burkholderiales
- Butyvibrio
- Paraprevotellaceae
- Erysipelotrichaceae
- Erysipelotrichi
- Erysipelotrichales
- Clostridia
- Catenibacterium
- Saprochaeta
- Saprochaetes
- Succinivibrio
- Aeromonas

**SELBAL analysis**

- Succinivibrio was associated with fermentation from vegetables and diets with high fiber amounts*
Results

The PREDIMED Trial in HIV individuals

By Adherence
punctuation
Relative abundances

MICROBIOME

Enriched bacterial genus depending on the PREDIMED Adherence Score improving

-2 -1 0 1 2 3
LDA SCORE (log 10)

Individuals who improve their diet habits based on the PREDIMED score had enriched in Bifidobacterium genus.

In that individuals, Bifidobacterium genus correlates with tolerogenic CD4 T-cell populations: TregCD25bright (P < 0.05; R2 = -0.55).

Spearman correlation with Holm’s correction (psych package - R software)
Conclusions

- Individuals who enrolled into Supplemented Mediterranean Diet (SMD) present changes in total cholesterol and LDL lipoprotein. But when we study the microbial population across the intervention group, we did not see major changes in the most abundant gut bacteria proportions.

- This study evidenced the importance of consider the Adherence score in the EVOO and nuts consumption in diet studies for HIV-infected individuals. We found strongly differences in the bacterial flora depending on it. As observed before in general population (*MC. Collado et al.).

- The Prevotella/Bacteroides ratio were lower in individuals which Adherence Score were less than 6 points, compared with the other Adherence groups.

- Individuals with High Adherence Score presented an enrich in the Succinivibrio genus. It was associated with the vegetables fermentation and diets with high contents of fiber and polysaccharides.

- Individuals who improved more than 3 points in the PREDIMED score present more Bifidobacterium at the end of the study; as we found this could be inverse relationship with inmuno tolerance.

*MC Collado et al. Front Microbiol. 2018