Metaboloma e Microbioma:

Approcci omici integrati per lo studio delle patologie endocrino-metaboliche

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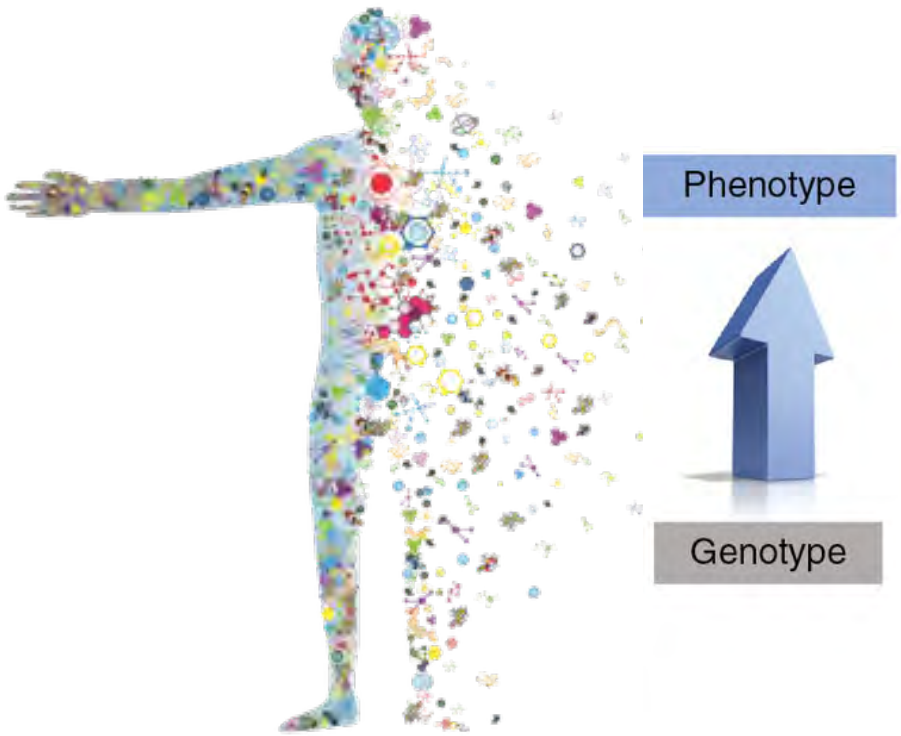
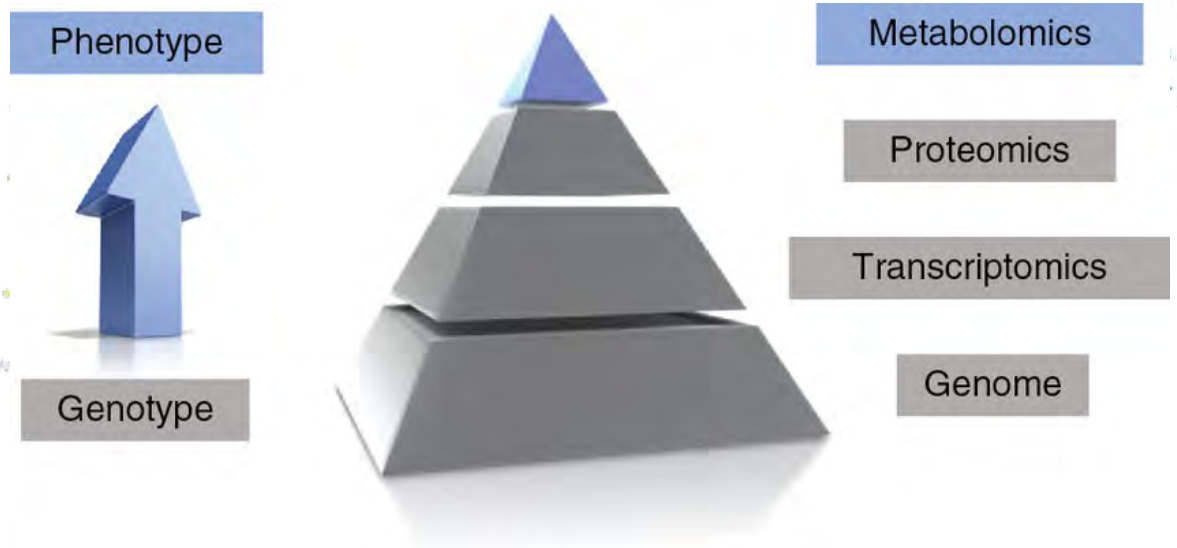
IL PRESENTE MATERIALE È RISERVATO AL PERSONALE DELL'UNIVERSITÀ DI BOLOGNA E NON PUÒ ESSERE UTILIZZATO AI TERMINI DI LEGGE DA ALTRE PERSONE O PER FINI NON ISTITUZIONALI

"I am large, I contain multitudes" (Walt Whitman)





Homo sapiens sapiens

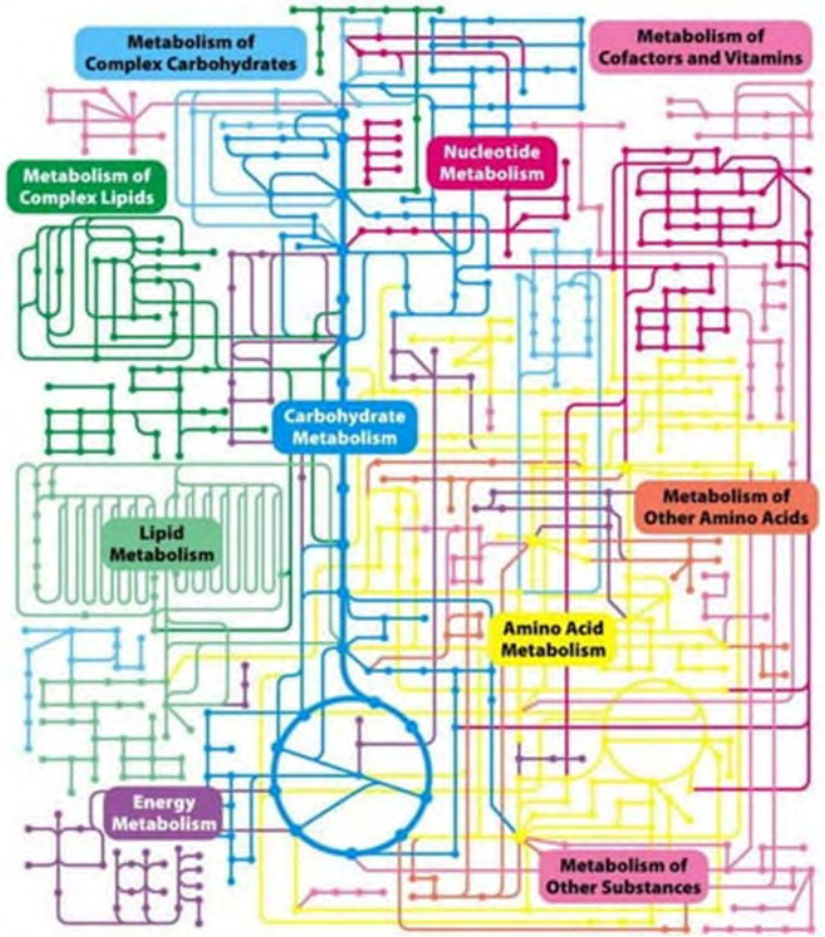
Holobiont

METABOLOMICS IN HUMAN HEALTH STUDIES

METABOLOME

The totality of low molecular weight compounds pertaining to all possible pathways that can be found in a defined cell, biological fluid or tissue.

The HMDB contains 220945 metabolite entries (accessed nov 2023).



METABOLOMICS

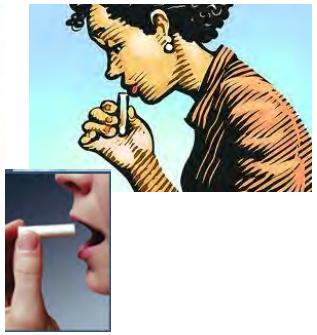
The **qualitative** and **quantitative** description of the metabolome.

Blood

Urine

Faeces

Dried blood spot



Saliva

MASS SPECTROMETRY-BASED ANALYTICAL STRATEGIES

TARGETED APPROACHES

↓

ESTABLISHED BIOMARKERS; HYPOTHESYS-DRIVEN INVESTIGATIONS

↓

QUANTITATION OF A PRE-SELECTED NUMBER OF COMPOUNDS

UNTARGETED APPROACHES

↓

LOOKING FOR UNKNOWN; NOT HYPOTHESYS-DRIVEN INVESTIGATIONS

↓

IDENTIFICATION OF IDEALLY ALL COMPOUNDS PRESENT IN A SAMPLE («omics»)

Since 2007…

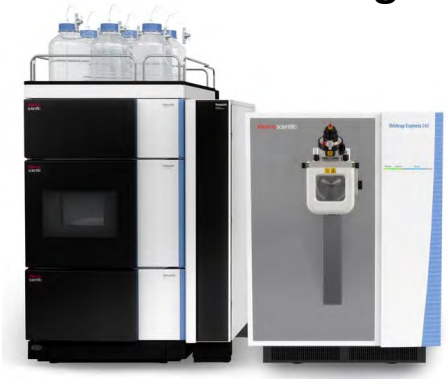
LC-MS/MS

API 4000 QTrap

Starting…

LC-HRMS

Orbitrap Exploris 240

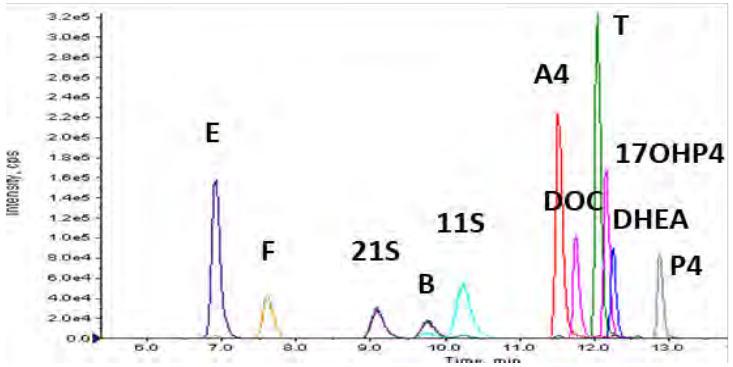


TARGETED LC-MS/MS PANELS (since 2007…)

Development, validation, reference interval generation, application to clinical studies

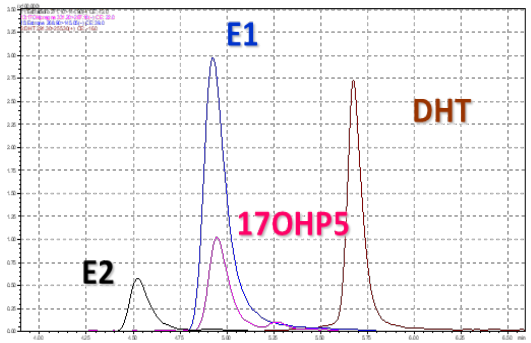
API 4000 QTrap

Panel 1: 11 serum steroids



Fanelli et al., Steroids 2011

Panel 2: 4 serum steroids

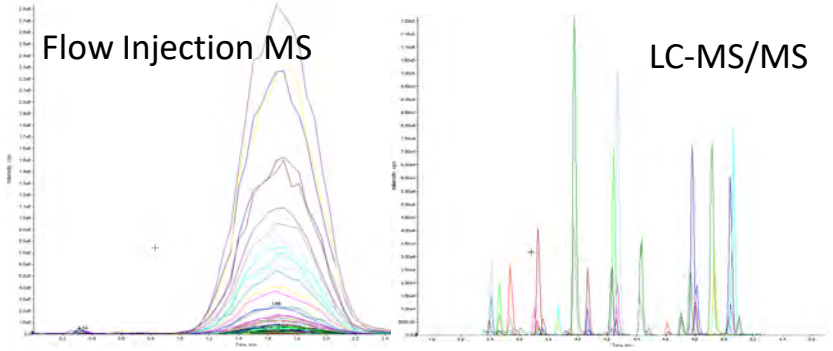


Mezzullo et al., SBMB 2020

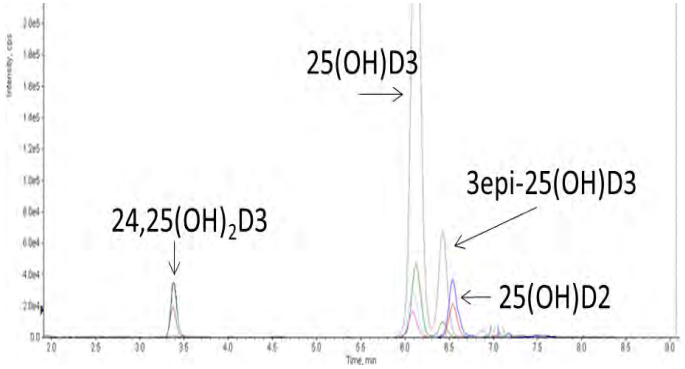
Panel 5: Targeted metabolomic profile (180 compounds)

BIOCRATES LIFE SCIENCES

- Acylcarnitines (40)
- Amino Acids (19 proteinogenic + Citrulline + Ornithine)
- Biogenic Amines (19)
- Hexoses (sum of Hexoses - 90 to 95% Glucose)
- Phospho- and Sphingolipids
  - Phosphatidylcholines (76)
  - Lyso-Phosphatidylcholines (14)
  - Sphingomyelins (15)

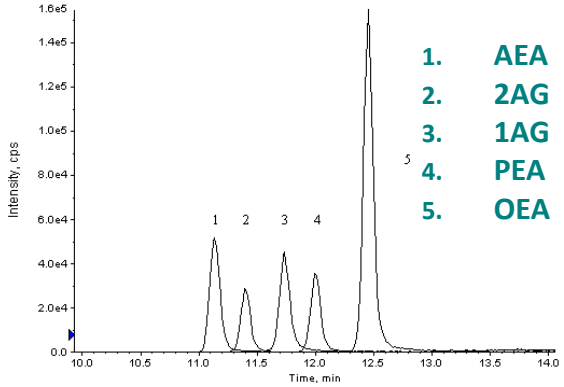


Panel 3: Vitamin D profile



Mezzullo et al., unpublished

Panel 4: Endocannabinoid profile



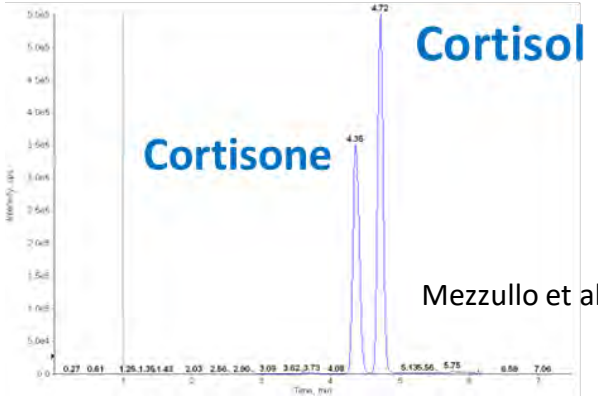
Fanelli et al., JLR 2012

TARGETED LC-MS/MS PANELS (since 2007…)

Development, validation, application to clinical and in vitro studies

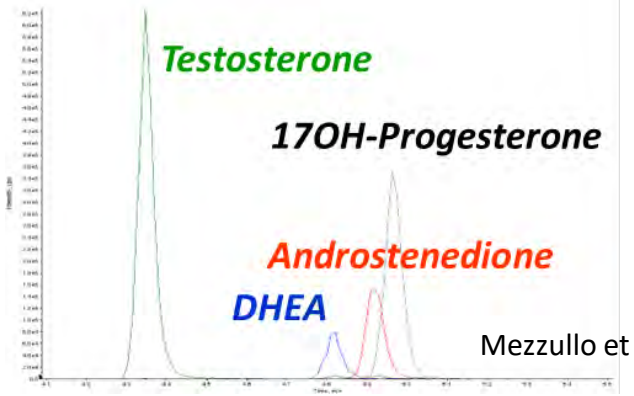
API 4000 QTrap

Panel 6: Salivary glucocorticoids

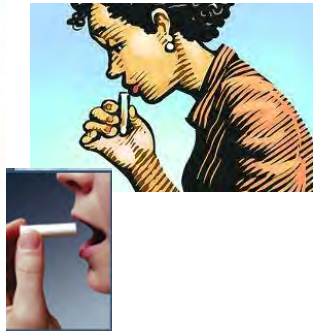


Mezzullo et al., SBMB 2016

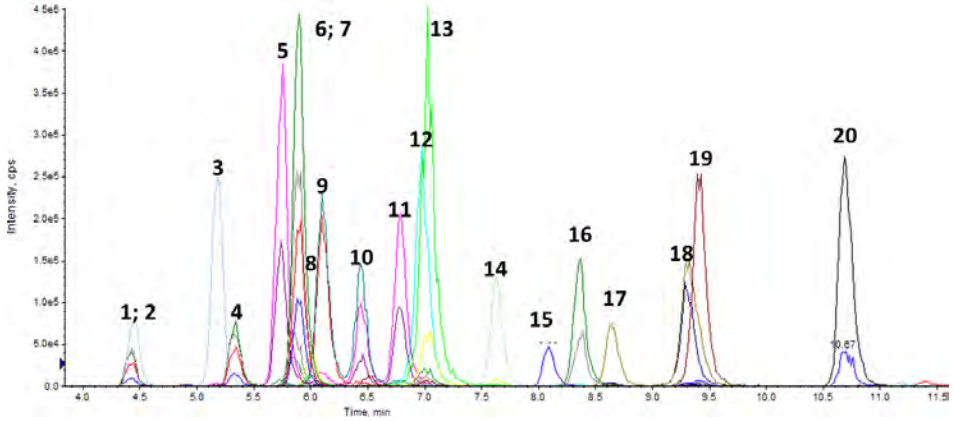
Panel 7: Salivary androgens



Mezzullo et al., CCLM 2017



Panel 8: 20 steroids from Leydig cells – Canonical and backdoor androgen pathways

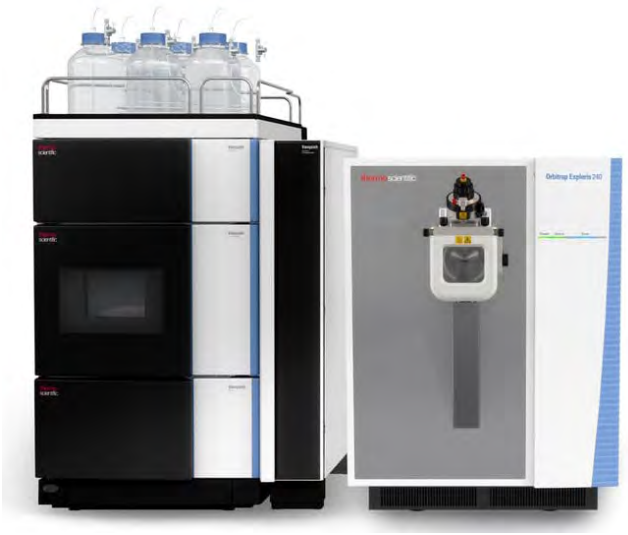


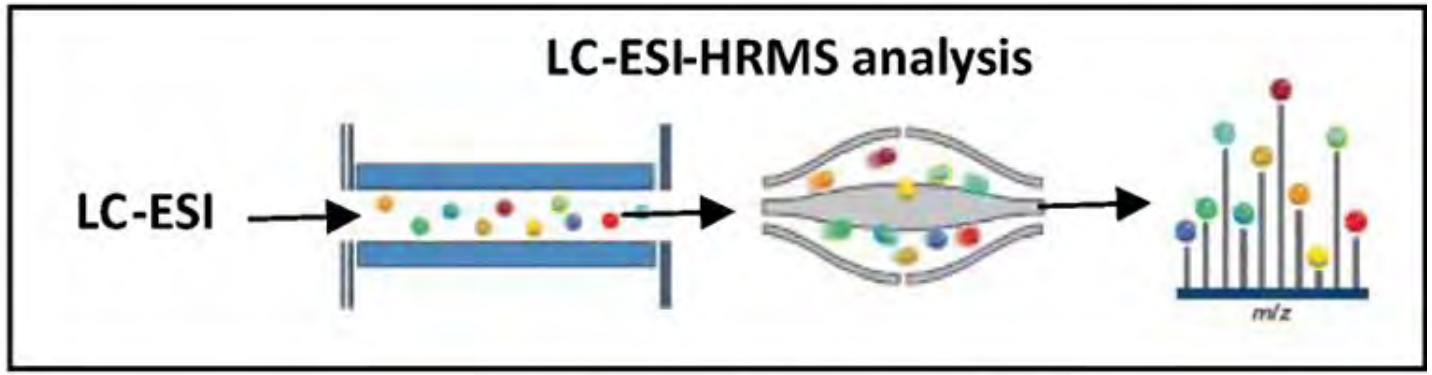
Fanelli et al., SBMB 2023

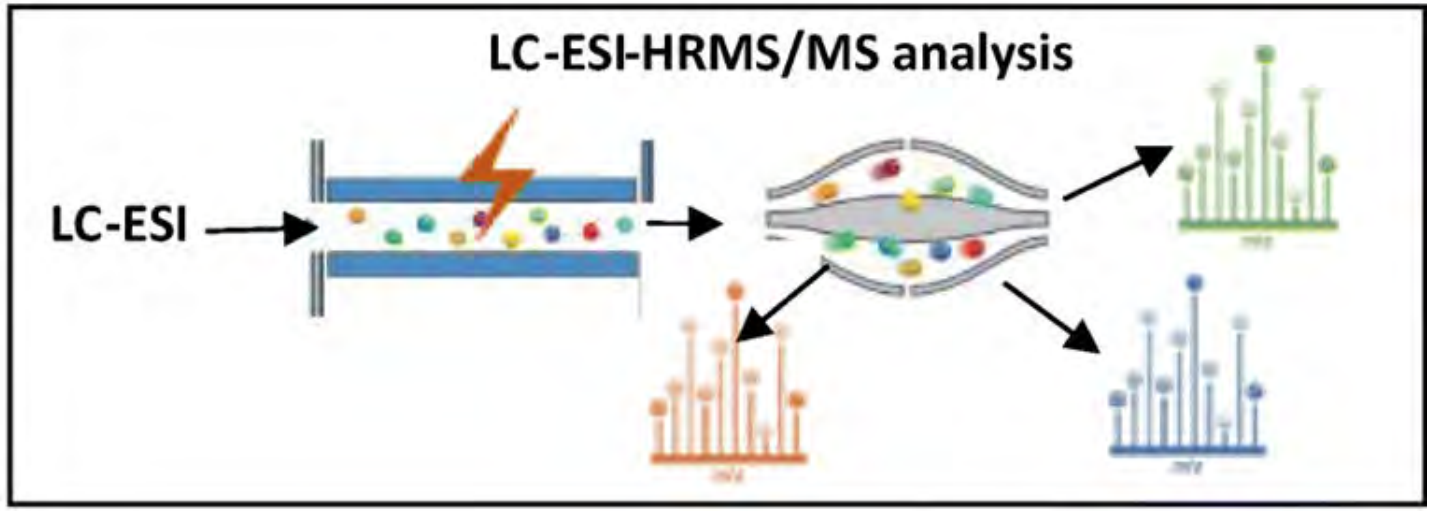
1 16OH-Progesterone (16OH-P4)
2 11-Deoxycortisol (11-S)
3 Androstenedione (A4)
4 11-Deoxycorticosterone (11-DOC)
5 Testosterone (T)
6 Androstenediol (A5)
7 17OH-Progesterone (17OH-P4)
8 17OH-Pregnenolone (17OH-P5)
9 Dehydroepiandrosterone (DHEA)
10 Androstanedione (5α-dione)
11 Epitestosterone (Epi-T)
12 Dihydrotestosterone (DHT)
13 17OH-Dihydroprogesterone (17OH-DHP4)
14 Progesterone (P4)
15 Androstanediol (3α-diol)
16 Androsterone (AN)
17 Pregnenolone (P5)
18 Dihydroprogesterone (5α-DHP4)
19 17OH-Allopregnanolone (17OH-Allo)
20 Allopregnanolone (Allo)

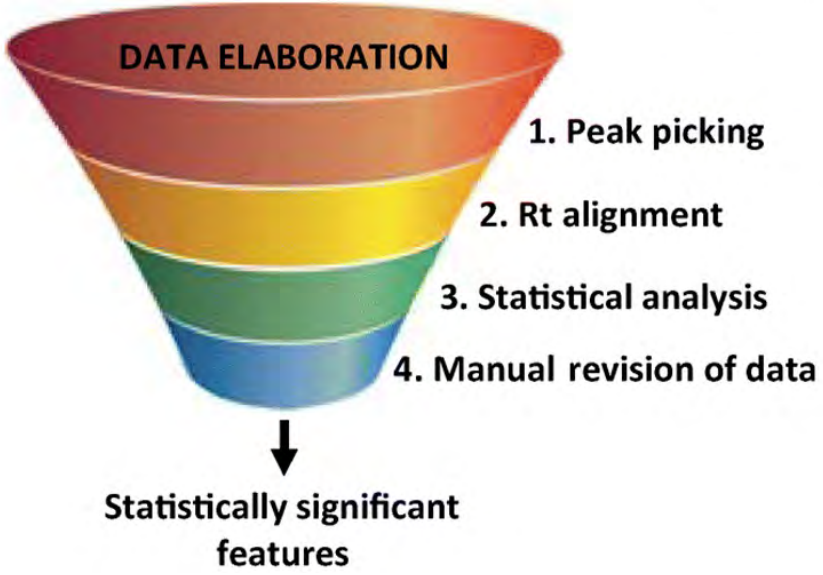
LIQUID CHROMATOGRAPHY – HIGH RESOLUTION MASS SPECTROMETRY FOR METABOLOMICS INVESTIGATION

Orbitrap Exploris 240









OMICs FACILITY

NMR-METABOLOMICS UNIT (DISTAL)
Prof Francesco Capozzi, Dr. Carlo Mengucci

LC-MS METABOLOMICS UNIT (DIMEC)
Prof. Uberto Pagotto, Dr. Flaminia Fanelli

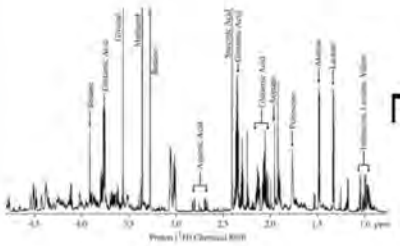
MICROBIOMICS UNIT (DIMEC)
Prof. Patrizia Brigidi, Dr. Monica Barone

Metabolomic Data Collection and Analysis

Blood | Urine | Faecal Matter

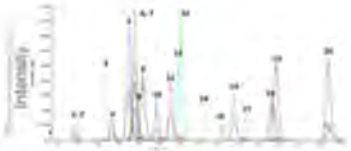
Experiments & Analysis (NMR Spectrometry)

- 600 MHz 1H NMR spectroscopy of biofluids and complex biological mixtures, high throughput
- Untargeted experiments, acquisition of the complete omic profile of a sample

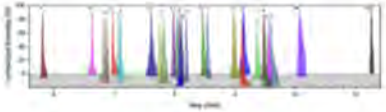


Experiments & Analysis (Mass Spectrometry)

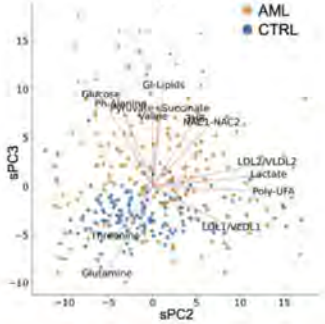
- LC-MS/MS for targeted quantification of a hypothesis-driven set of compounds



- LC-HRMS for untargeted experiments, acquisition of the complete omic profile of a sample



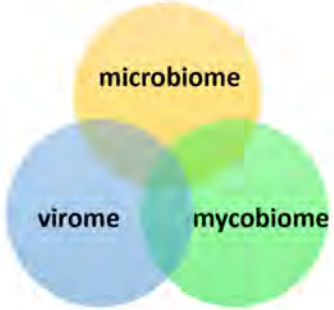
Molecular Information Deconvolution



Microbiomic Data Collection and Analysis

Faecal Matter

Phylogenetic and functional characterization of human microbes from a wide range of biological samples



Experiments and Analysis

Target Gene Approach (Bacteria, Archaea, Fungi)

16S rRNA, 18S, ITS gene

Aim: Phylogenetic Composition

Shotgun Approach (All Microbes)

- **Metagenomics for putative functional pathways**
- **Metatranscriptomics for active pathways and microorganisms**

WORKFLOW FOR BIOMARKER DISCOVERY

Multi-omics and clinical data integration to feed Machine Learning algorithms

OMICs FACILITY

MICROBIOMICS UNIT (DIMEC)
Prof. Patrizia Brigidi, Dr. Monica Barone
NMR-METABOLOMICS UNIT (DISTAL)
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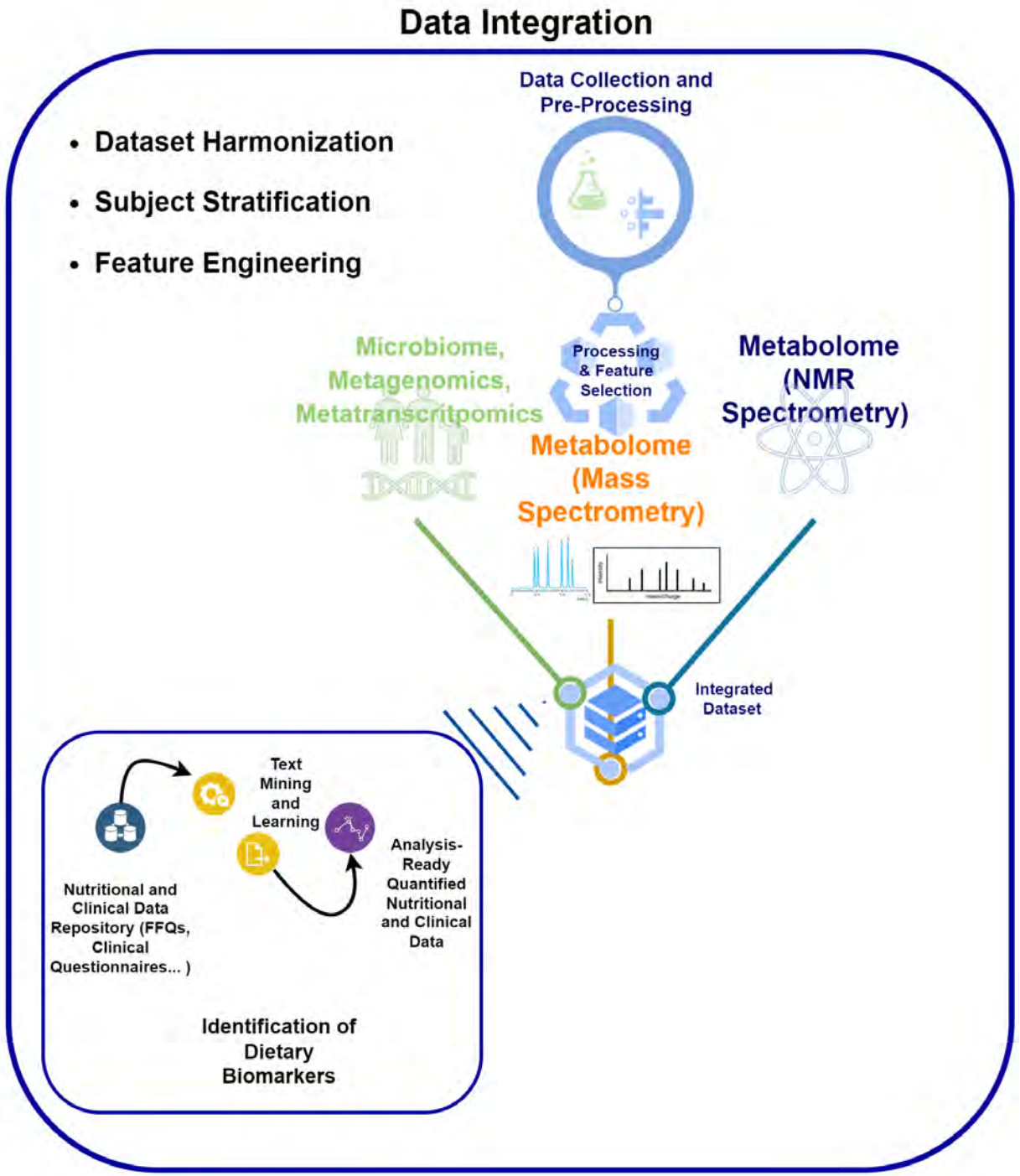
LC-MS METABOLOMICS UNIT (DIMEC)
Prof. Uberto Pagotto, Dr. Flaminia Fanelli

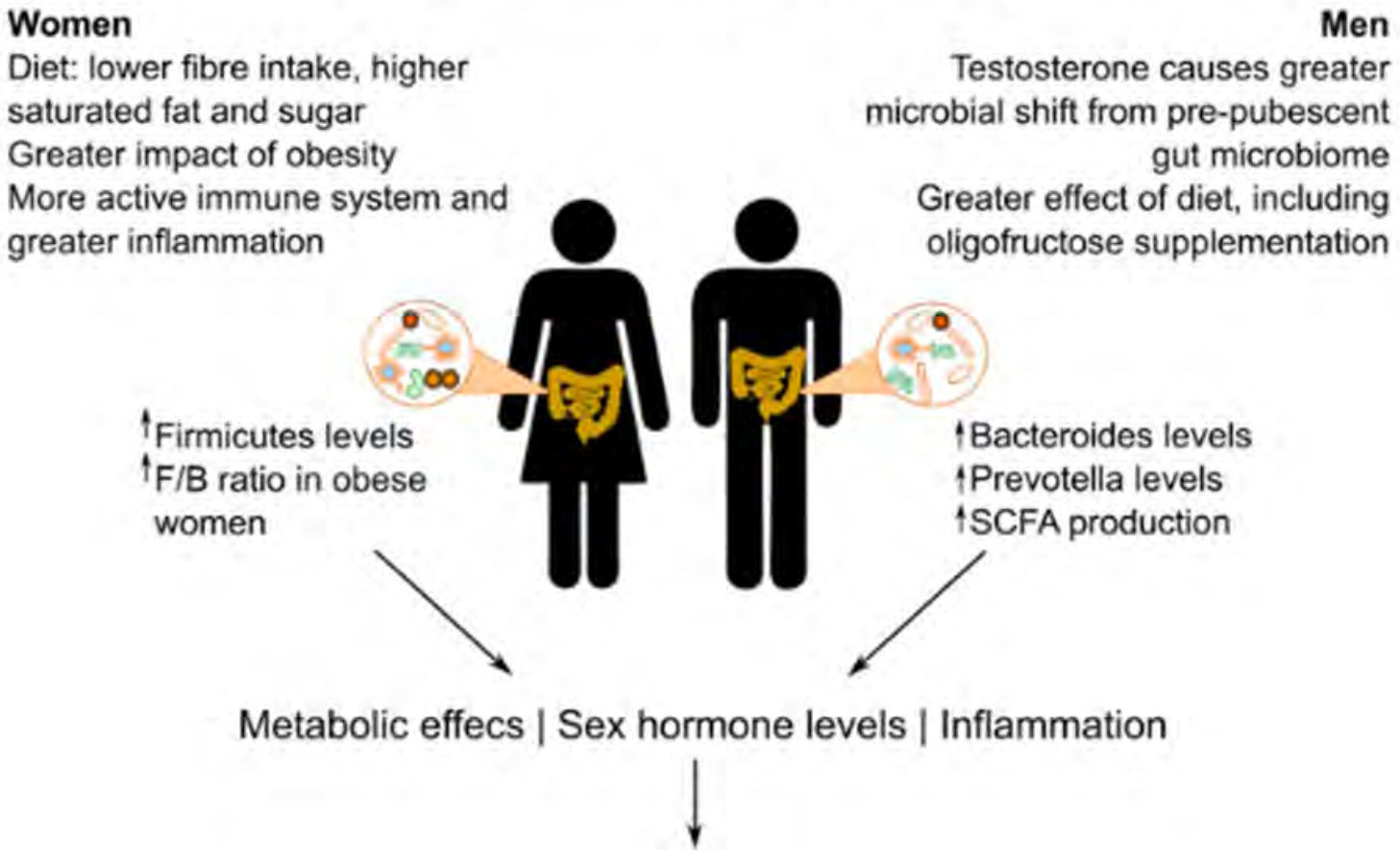
CLINICAL UNIT

Unit of Prevention and Care of Diabetes, IRCCS Policlinico S.Orsola - DIMEC, University of Bologna
Prof. Uberto Pagotto and team



MICROBIOTA – HOST INTERACTION IN ENDOCRINE SYSTEMS

Sex differences in determinants, constituents and effects of the gut microbiome

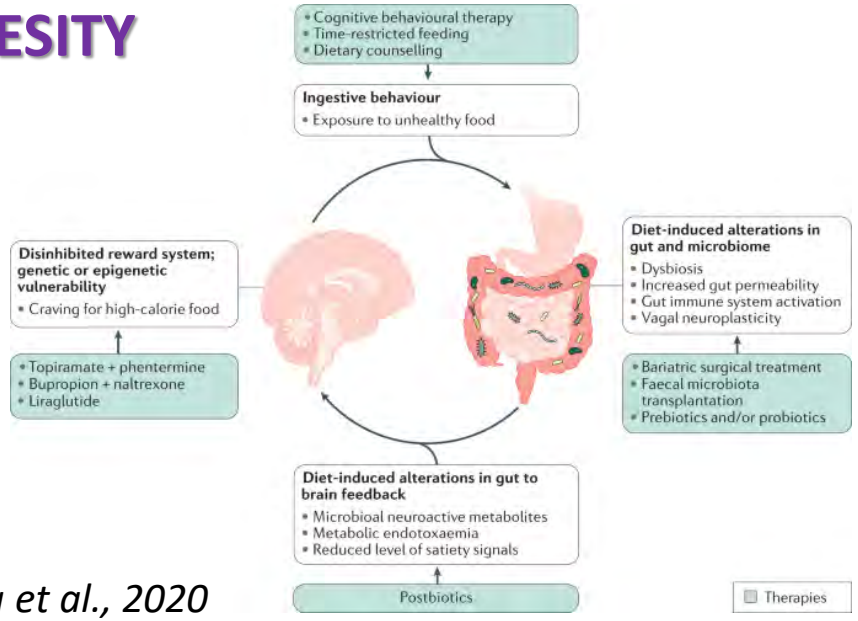


Beale et al., 2019

FEMALE HYPERANDROGENISM and MALE HYPOGONADISM

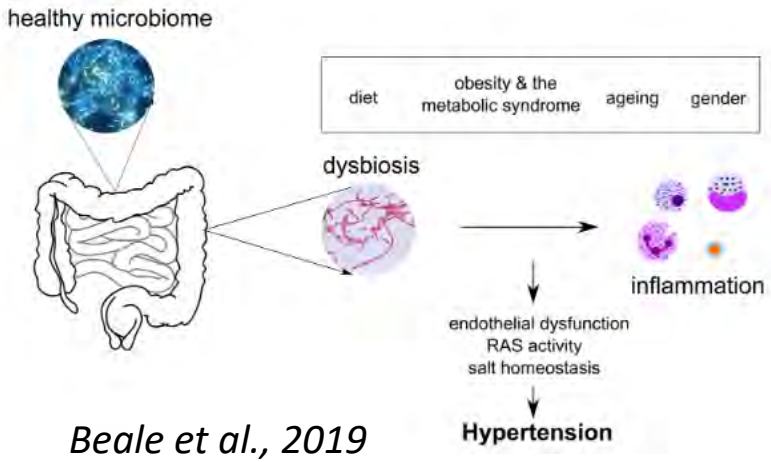
MICROBIOTA – HOST INTERACTION IN ENDOCRINE SYSTEMS

OBESITY

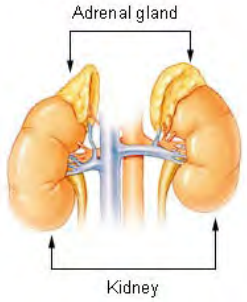


Gupta et al., 2020

HYPERTENSION – ADRENALS

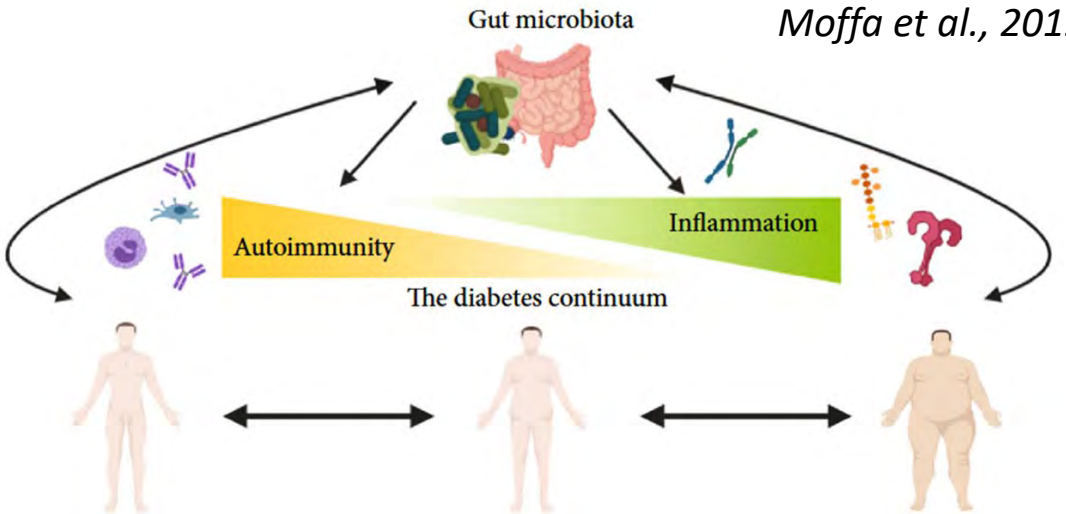


Beale et al., 2019

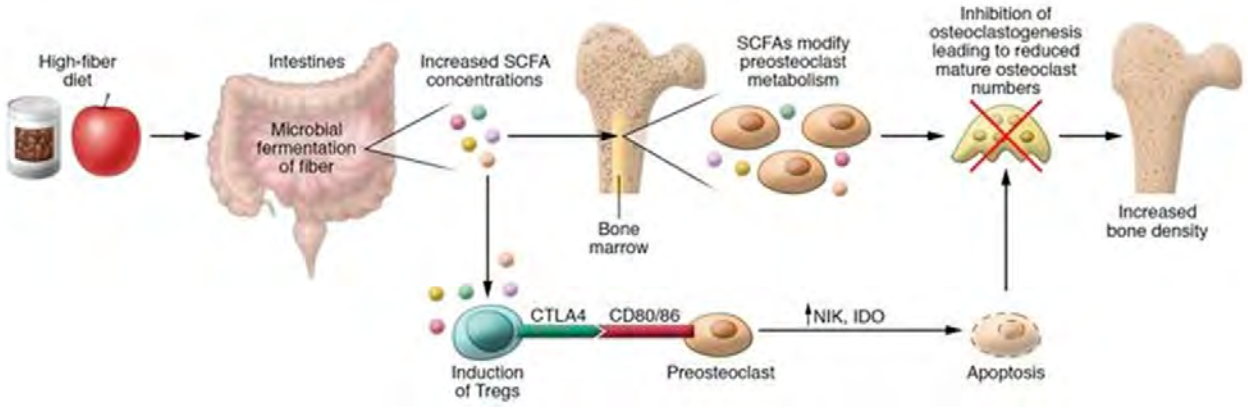


DIABETES

Moffa et al., 2019



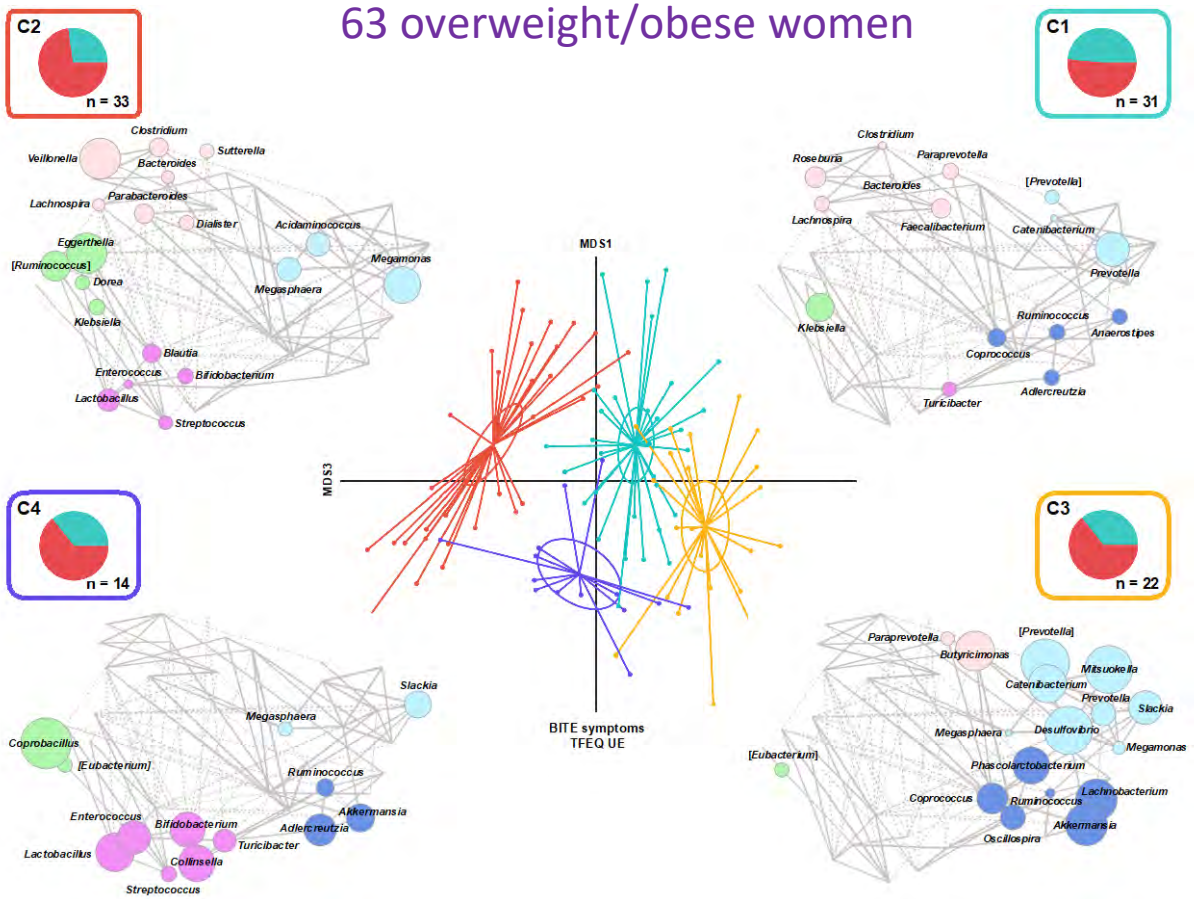
BONE REMODELING



Zaiss et al., 2019

BRAIN–GUT–MICROBIOME INTERACTIONS IN OBESITY AND EATING BEHAVIOUR

37 normal-weight and
63 overweight/obese women



Barone et al., 2022

European FP7 projects

NeuroFAST

My New GUT

Metagenomic and metatrascriptomic signatures

Microbiome compositional clusters associated with:

BMI and metabolic profile
Nutritional choiches
Eating behaviour – food addiction

Identification of clusters featured by altered transcription of microbial genes involved in peripheral and central biochemical pathways:

Bile acids metabolization
Detoxiphication and morphine metabolism
Genes involved in endocannabinoid and GABA synthesis

4DI: «Different Diets for Different Diabetes»

POS5 – IDENTITA

Puglia Salute

Patients affected by <u>Type II Diabetes</u> from

- *Italy*
- *North Africa*
- *South-east Asia*
- *East Europa*

Longitudinal monitoring for 12 months

Dietitian

Personalized mediterranean diets

Endocrinologist

Monitoring of Diabetes and dysmetabolism.

Chef

Recipies based on personalized diets and on ethnic tradition.

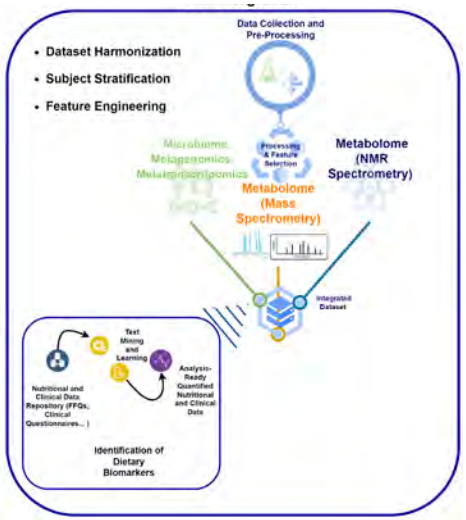
Personal trainer

Assisting patients during group walks and monitoring improvements

DBS

Saliva

Multi-omics and clinics integration



Objectives:

- Evaluate the glycaemia control of the personalized diet regimen in addition to physical activity and pharmacological therapy (according to guidelines) vs current guidelines nutritional and behavioral approaches (HbA1c, BMI, waist circumference).

- To Identify metabolic and steroid markers associated with, and predictive of, the effectiveness of the nutritional therapy in improving the glycaemia and metabolic control.

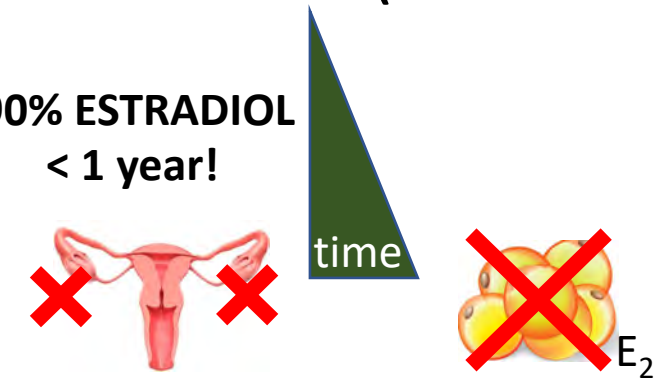
PNRR PE10 – Spoke 5

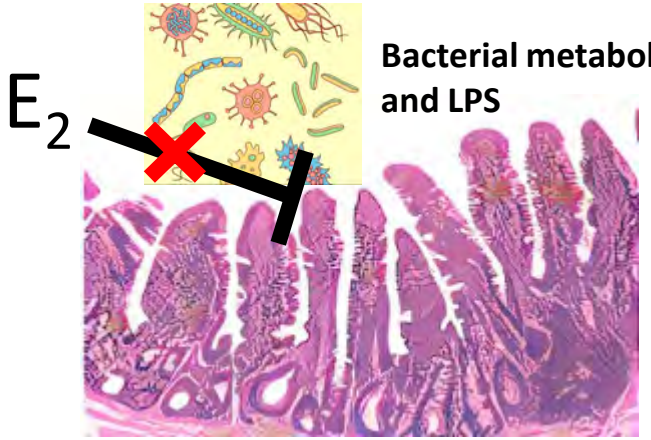
ON FOODS

Multidimensional characterization of breast cancer patients receiving **adjuvant hormone blockade therapy** evaluated for bone mineral metabolism and subjected to metabolomic and intestinal microbiota analysis.

IATROGENIC MENOPAUSE (aromatase inhibitors ±GnRH agonists)

↓90% ESTRADIOL
< 1 year!

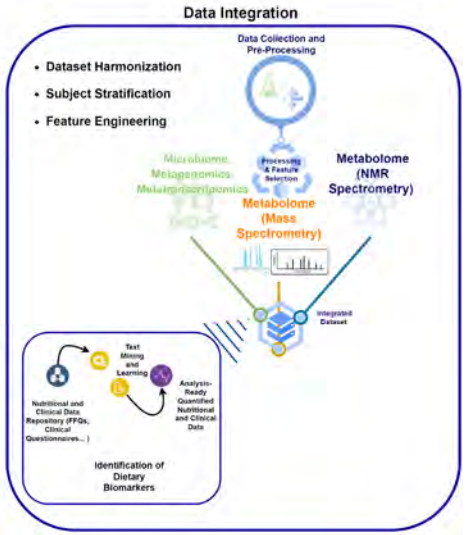




Marked increase in pro-inflammatory and osteogenic cytokines, RANKL, ↓ 5-hydroxytryptamine

- Damage-induced increase in intestinal barrier permeability
- Pro-inflammatory status

Multi-omics and clinics integration



Objectives

- Identify early endocrine-metabolic-nutritional-microbial biomarkers for pre-therapy risk stratification.
- Identify biomarkers of accelerated antiestrogenic therapy-induced bone loss.
- Develop algorhithm for the effective monitoring of bone health during and following antiestrogenic therapy.

Centro unificato di Ricerca Biomedica applicata

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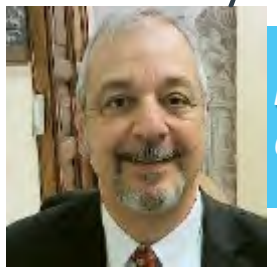

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