Brief Summary of the collaborative Project Entitled:

METABOLOMIC STUDY OF DISCORDANT PHENOTYPES OF OBESITY AND TYPE 2 DIABETES

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The MHO Paradox

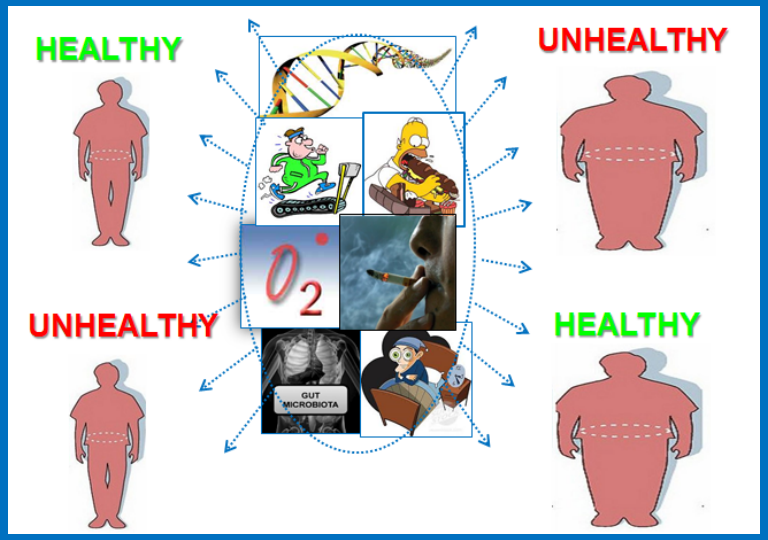
~ 25% Morbid Obese does NOT devolop IR/T2D
~ 18% Not-Obese DOES develop IR/T2D

OBESITY

To be obese—does it matter if you are metabolically healthy?

Antony D. Karelis

nature REVIEWS ENDOCRINOLOGY



What Makes The Difference?

HPs - OBJs

The Blood and VAT Metabolome Contain Relevant Biological and Diagnostic Information

- **To Identify Novel Biomarkers Able to Discriminate Discordant Phenotypes of Obesity/T2D from the 'Diabese' Phenotype and the Controls, to Unveil New Etiopathogenic mechanisms linking the two conditions**

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❖ WHAT DO WE HAVE ongoing? Study Designs & Samples Available (FIS-SAS)

Discordant Phenotypes: Observational STUDY

BMI (SEEDO)	pre T2D-T2D: NO	pre T2D-T2D: YES
	GROUP 1	GROUP 4 (DISC. PHENOTYPE A)
	GROUP 2	GROUP 5
	GROUP 3 (DISC. PHENOTYPE A)	GROUP 6

✓ Biosamples:

n=155

S — n = 155

VAT — n = 128

P — n = 25 PILOT*

*3 groups; not exactly the same subjects

Gastric Bypass Follow-Up STUDY

Time (months)

pre T2D-T2D	T0	T1	T3	T6
NO	T0	T1	T3	T6
YES				

✓ n= 40 volunteers x 4 Timepoints
✓ Biosamples:

S — n = 142 (some timepoint is missing)

Study Design

Observational STUDY

- ✓ Subjects recruited from Malaga Hospital Complex (Málaga, Spain); Informed consent; Ethical Committee aprroval
- ✓ INCLUSION Criteria / Grouping:

	Pre-diabetes or Diabetes: Hyperglycemia (fasting plasma glucose ≥ mg/dL) Insulin Resitance (HOMA-IR > 3.4)	
Body Mass Weight (BMI)	**NO**	**YES**
Normal Weight & Grade I Overweight (BMI <27)	**GROUP 1**	**GROUP 4 (DISC. PHENOTYPE A)**
Grade I & II Obesity (BMI 30 - 40)	**GROUP 2**	**GROUP 5**
Morbid Obesity (BMI >40)	**GROUP 3 (DISC. PHENOTYPE A)**	**GROUP 6**

- ✓ Sampling: n = aprox 30/group (sex- and age-matched)
- ✓ Biosamples: Blood Serum, Blood Plasma (pilot); Visceral Adipose Tissue
- ✓ EXCLUSION for: T2D medication, acute Inflammatory and Infective disease, alcohol & drug abuse

Potential Issues:

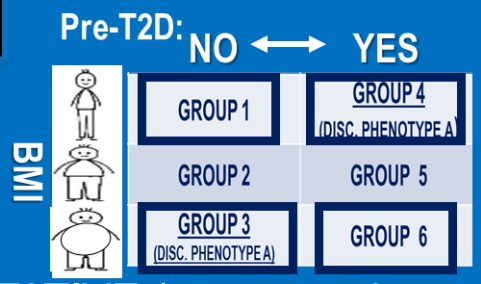
Treatment of confounders (MULTIdrugs, MULTIdiets)

Management of complexities (MULTIclass analysis by MVA)

Sampling: n < in Discordant Phenotypes groups. Rare phenotypes

Discordant Phenotypes: Observational STUDY

TARGETED APPROACH: Metabolic Profiling

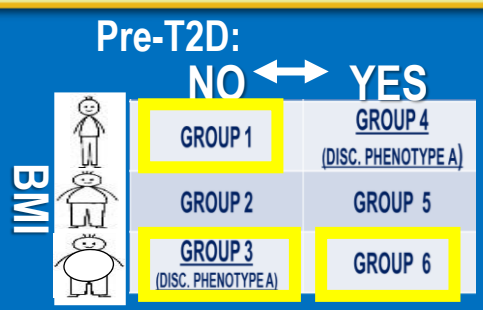


≠ subjects
≠ samples

- ✓QUANTITATIVE (concentrations nM – µM)
- ✓**81** POLAR metabolites (Amino Acids, Biogenic Amines, Acylcarnitines, Total Hexoses)
- ✓**399** NON-POLAR metabolites (Glycerophospholipids, Sphingolipids, Ceramides)
- ✓Randomized sequence, QCs (30%)
- ✓No replicates
- ✓Metabolites filtered out if: a) CV>25% in QCs; b) >15% below LOD*

*McNewmar's test to compare the proportion of detectable values for discarded metabolites???

UNTARGETED APPROACH Metabolic Fingerprinting



- ✓ NOT QUANTITATIVE : mass signal intensities
- ✓ 5000 mass signals (m/z, RT) * ESI +/-
- ✓ Randomized sequence, QCs (30%)
- ✓ ISmix, ESmix. Apparent no need for normalization
- ✓ Low intensity signals:
- ✓ Noisy signals filtered out : excluded those that were NOT positively detected in at least the 40% of each of the study classes

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High-throughput Transversal technologies
-METABOLOMICS PLATFORM-

UNTARGETED APPROACH
Metabolic Fingerprinting

- ✓ Hypothesis-GENERATING
- ✓ EXPLORATIVE, BIOMARKERS DISCOVERY
- ✓ MVA, Phenotype-Metabolome Associations
- ✓ IDEALLY unprocessed samples
- ✓ Exact Mass

LC-ESI-QToF-MS (QSTAR Elite)

TARGETED APPROACH:
Metabolic Profiling

- ✓ Hypothesis-DRIVEN → only a cluster of metabolites
- ✓ QUANTITATIVE, BIOMARKERS VALIDATION
- ✓ UVA, ROC curves
- ✓ Pre-chromatographic treatment
- ✓ High sensitivity

LC-ESI-QqQ-MS/MS (MRM, PrIS, NL)

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