

## **Reviews in Endocrine and Metabolic Disorders**

**Title: Metabolomic signatures after bariatric surgery – a systematic review**

**Authors:** Matilde Vaz<sup>1,2\*</sup>, Sofia S. Pereira<sup>1,2\*</sup>, Mariana P. Monteiro<sup>1,2</sup>

<sup>1</sup> Endocrine & Metabolic Research, Unit for Multidisciplinary Research in Biomedicine (UMIB), University of Porto, Porto, Portugal.

<sup>2</sup> Department of Anatomy, School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal.

\* Matilde Vaz and Sofia S. Pereira have contributed equally to this work.

**Corresponding Author:** Mariana P. Monteiro (mpmonteiro@icbas.up.pt)

## Supplementary File 5 - Summary of studies comparing the metabolomic profiles of patients with different outcomes (T2D and weight loss)

| FIRST AUTHOR AND YEAR | EXPERIMENTAL DESIGN      | SURGICAL PROCEDURE | SUBJECTS                                                       | PRE- OPERATIVE BMI (kg/m <sup>2</sup> ) | AGE AT SURGERY (YEARS) | FEMALE: MALE | POST-OPERATIVE BMI (kg/m <sup>2</sup> )          | BIOLOGICAL SAMPLE     | METABOLOMIC ANALYSIS | OUTCOMES AND MAIN FINDINGS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-----------------------|--------------------------|--------------------|----------------------------------------------------------------|-----------------------------------------|------------------------|--------------|--------------------------------------------------|-----------------------|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Abidi W, 2020 [55]    | Prospective cohort study | RYGB               | Control group - patients with obesity (N=11)                   | 47.7 ± 8.6                              | 42.0 ± 11.1            | 1:1          | Nadir BMI: 28.2 ± 4.8<br>Current BMI: 29.4 ± 4.6 | Serum fasting samples | LC-MS                | <p>Outcomes: <b>Patients with sustained weight loss</b> at 5.0 ± 3.7 years (n=14) and <b>patients with weight regain</b> at 8.9 ± 3.0 (n=21)</p> <p><u>Patients with sustained weight loss:</u><br/>↓ metabolites of fatty acids metabolism, including TGs, DGs and CEs;<br/>↑ 3-HB and glycine</p> <p><u>Patients with weight regain:</u><br/>↓ metabolites related to serine, glycine and threonine pathway, phenylalanine metabolism, TCA cycle, and alanine and glutamate metabolism; ↑ other AAs.</p> |
|                       |                          |                    | Patients with obesity undergoing RYGB (N=35)                   | 49.9 ± 7.3                              | 47.9 ± 10.7            | 20:1         | Nadir BMI: 28.9 ± 2.9<br>Current BMI: 37.9 ± 5.7 |                       |                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Ahlin S, 2019 [46]    | Prospective cohort study | RYGB + BPD         | Patients with obesity (N= 15), in which 13,3% with T2D (N= 2)  | 51.6 ± 9.6                              | 44.3 ± 8.3             | 1:2          | 37.7 ± 7.4                                       | Fasting plasma        | Targeted UPLC-MS     | <p>Outcome: Patients with obesity and improved IR after a mean of 185.3 (72.9) days</p> <p>↑ total BAs concentrations after RYGB and BPD.</p>                                                                                                                                                                                                                                                                                                                                                              |
| Arora T, 2015 [25]    | Prospective cohort study | RYGB               | Patients with obesity (N= 16), in which 87.5% with T2D (N= 14) | 51.0±2.3                                | 48.1±2.3               | 3:4          | Reduction of 30.5 ± 4.4 %                        | Fasting plasma        | GC-MS and UPLC-MS    | <p>Outcomes: <b>patients with</b> (n=7) or <b>without</b> (n=7) T2D remission at 2 years</p> <p><u>Patients with T2D remission:</u></p> <p>Before surgery, ↑ metabolites from TCA cycle and pentose phosphate pathways, TG and PLs with LCFAs, and PEs.</p> <p>After 4 days, ↑ aconitate, indole acetic acid and ribitol; ↓ most lipid species.</p> <p>After 42 days, ↑ decanoic and octanoic acids; ↑ most lipid species.</p>                                                                             |
|                       |                          |                    |                                                                | 47.2±1.6                                | 48.8±3.5               | 7:0          | Reduction = 32.0 ± 2.3 %                         |                       |                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Ha J, 2020 [62]       | Prospective cohort study | RYGB + SG          | Patients with obesity <sup>s</sup> and T2D (N= 24)             | 39.6 ± 7.9                              | 45.4 ± 10.2            | 17:7         | 28.8 ± 6.6                                       | Fasting serum samples | Targeted LC-MS       | <p>Outcomes: <b>patients with</b> (n=14) or <b>without</b> (n=10) T2D remission at 1 year</p> <p><u>Patients with T2D remission:</u></p> <p>↑ baseline levels of L-DOPA and 3-HAA (sustained up to 3 months after surgery).</p> <p>At 1 year, no differences in the levels of L-DOPA and 3-HAA between both outcomes.</p> <p>No distinction between both surgery procedures.</p>                                                                                                                           |
|                       |                          |                    |                                                                | 33.9 ± 4.5                              |                        |              | 28.9 ± 5.6                                       |                       |                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

|                     |                          |            |                                                             |                                         |                                  |       |                                                    |                                    |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|---------------------|--------------------------|------------|-------------------------------------------------------------|-----------------------------------------|----------------------------------|-------|----------------------------------------------------|------------------------------------|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Kindel T, 2018 [49] | Prospective cohort study | SG         | Patients with obesity (N= 28), in which 21% with T2D        | 45.0 ± 6.8                              | 45.4 ± 12.9                      | 41:9  | At 6 weeks: 40.7 ± 6.1<br>At 12 weeks: 38.9 ± 6.3  | Fasting/postprandial serum samples | UPLC-MS         | <p>Outcome: Patients with significant weight loss</p> <p>SG induced an early (6 weeks after) and persistent (12 weeks after) ↑ in multiple BAs subtypes:</p> <p>-fasting HCA (total, unamidated and G- sub-fractions).</p> <p>- post-prandial BAs (total and G-), CDCA (total and G-), DCA (total and G), MCA (total and G-), HCA (total, unamidated and G-).</p> <p>↑ postprandial total and G-CDCA was significantly correlated to the 6 weeks BMI loss; ↑ post-prandial G-HCA was significantly correlated to increased weight loss at both 6 and 12 weeks.</p> |
| Kwon H, 2014 [63]   | Prospective cohort study | RYGB + DJB | Patients with obesity and T2D (N= 22)                       | 30.8 ± 5.6                              | 39.8 ± 9.2                       | 1:1   | 27.2 ± 3.0                                         | Plasma samples                     | GC-MS and H-NMR | <p>Outcomes: Patients with (n=10) or without (n=12) HbA1c improvement at 3 months</p> <p>After 1 week, ↑ 3-HB in the improved group, whereas ↑ glucose and lipids (LDL, VLDL) in the non-improved group.</p> <p>No distinction between both surgery procedures.</p>                                                                                                                                                                                                                                                                                                |
|                     |                          |            |                                                             | 24.6 ± 3.1                              | 49.8 ± 6.9                       | 7:3   | 21.7 ± 2.2                                         |                                    |                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Kwon Y, 2020 [54]   | Prospective cohort study | SG         | Patients with obesity (N= 27), in which 74% with T2D (N=20) | 38.7 ± 5.2                              | 42.1 ± 12.9                      | 17:10 | At 3 months: 31.5 ± 5.1<br>At 6 months: 27.9 ± 4.6 | Fasting serum samples              | Targeted LC-MS  | <p>Outcome: Patients with significant weight loss</p> <p>Before SG: ↓ serotonin and serotonin/5-HT<sub>1A</sub> ratio; ↑ 5-HIAA, 5-HIAA/serotonin ratio and BCAA (α-isoleucine), were significantly associated with greater WL% at 3 and 6 months after SG.</p>                                                                                                                                                                                                                                                                                                    |
| Kwon Y, 2021 [29]   | Prospective cohort study | SG         | Patients with obesity and T2D (N=23)                        | 38.9 ± 5.2                              | 41.8 ± 13.1                      | 17:6  | 31.4 ± 5.5                                         | Fasting serum                      | Targeted LC-MS  | <p>Outcome: Patients with obesity and T2D, but improved IR at 3 months</p> <p>↑ baseline KynP metabolites and ↓ after SG.</p> <p>↓ BCAAs and AAAs after surgery.</p> <p>↓ TDGMs after surgery.</p>                                                                                                                                                                                                                                                                                                                                                                 |
| Li QR, 2018 [61]    | Prospective cohort study | RYGB       | Patients with obesity (N=19), in which 47% with T2D (N=9)   | 41.5 ± 4.8 (no T2D)<br>43.1 ± 5.1 (T2D) | 43 ± 13 (no T2D)<br>52 ± 9 (T2D) | NA    | 31.0 ± 5.1 (no T2D)<br>33.9 ± 7.5 (T2D)            | Fasting and postprandial plasma    | LC-MS/MS        | <p>Outcome: Patients with obesity and no T2D (N=10) and patients with obesity and T2D, but improved IR at 12 months (N=9)</p> <p>After RYGB, ↓ fasting plasma BCAAs was associated with improved IR.</p>                                                                                                                                                                                                                                                                                                                                                           |
| Lopes T, 2016 [58]  | Prospective cohort study | RYGB       | Patients with obesity and T2D (N=10)                        | 32.38 ± 2.11                            | 25 to 65                         | 1:1   | 25.48 ± 1.85                                       | Plasma samples                     | H-NMR           | <p>Outcome: Patients with T2D remission at 1 year</p> <p>After RYGB: ↓ BCAAs.</p> <p>After RYGB in postprandial analysis: ↓ VLDL, LDL, N-acetyl glycoproteins and unsaturated lipids; ↑ HDL and PC.</p>                                                                                                                                                                                                                                                                                                                                                            |

|                               |                            |      |                                                                |            |                                    |       |                                                                      |                |                                    |                                                                                                                                                                                                                                                                                                                                                                                                         |
|-------------------------------|----------------------------|------|----------------------------------------------------------------|------------|------------------------------------|-------|----------------------------------------------------------------------|----------------|------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Luo P, 2016 [17]              | Retrospective cohort study | RYGB | Patients with obesity <sup>s</sup> and T2D (N=35)              | 30.8 ± 3.3 | 49.8 ± 9.9 (6months after surgery) | 19:16 | 24.3 ± 2.4                                                           | Serum samples  | Untargeted UPLC-MS                 | <p>Outcomes: Patients with (n=23) or without (n=12) T2D remission at 1 year</p> <p>Significant metabolic alterations related to FFA, acylcarnitines, AAs, BAs, and lipids species in both outcomes.</p> <p><u>Patients with T2D remission:</u></p> <p>↑ baseline levels of glycoconjugates, bilirubin and tryptophan.</p> <p>6 months after RYGB: ↓ FFA 16:0, FFA 18:3, FFA 17:2 and hippuric acid.</p> |
|                               |                            |      |                                                                |            |                                    |       | 24.2 ± 2.3                                                           |                |                                    |                                                                                                                                                                                                                                                                                                                                                                                                         |
| Narath SH, 2016 [20]          | Prospective cohort study   | RYGB | Patients with obesity (N= 44), in which 54,5% with T2D (N= 24) | 43.9 ± 5.4 | 46.8 ± 11.3                        | 29:15 | <p>At 1-3 weeks: 40.8 ± 5.2 (SD)</p> <p>At 1 year: 30 ± 4.4 (SD)</p> | Serum samples  | Untargeted LC-HRMS                 | <p>Outcomes: Patients with high or low WL at 1 year (above or below the median of the WL); patients with (N=15) or without (N=9) T2D remission at 1 year.</p> <p>After 1 year: ↓ creatine, ornithine, arginine and valine in patients with high WL; ↓ sarcosine, alanine, leucyl-proline and pyroglutamic acid in patients with T2D remission.</p>                                                      |
| Samczuk P, 2018 [57]          | Prospective cohort study   | SG   | Patients with obesity and T2D (N=20)                           | 49 ± 4.5   | 47 ± 10.3                          | 4:7   | 37 ± 3.6                                                             | Fasting serum  | Untargeted GC-MS and LC-MS         | <p>Outcomes: Patients with quicker (n=11) or slower (n=9) remission of T2D</p> <p><u>Quicker responders:</u></p> <p>↓ baseline LPC and LPE.</p> <p>Postoperative significantly changed metabolites can be classified mainly as PC, LPC, PE, LPE, AAs, organic acids, sugars and metabolites related to gut microbiota metabolism.</p>                                                                   |
|                               |                            |      |                                                                | 51 ± 8.3   | 51 ± 11.4                          | 4:5   | 43 ± 7.6                                                             |                |                                    |                                                                                                                                                                                                                                                                                                                                                                                                         |
| Shantavasi nkul PC, 2018 [56] | Prospective cohort study   | RYGB | Control group - patients with obesity in a WLMP (N=11)         | 38.2 ± 4.6 | 56.5 ± 11.4                        | 4:7   | Decrease of 7.5 ± 1.2                                                | Plasma samples | Non-targeted GC/MS and targeted MS | <p>Outcomes: Patients with weight loss and improved HOMA-IR after RYGB (N=11) and patients with similar weight loss after WLMP (N=11)</p> <p><u>Patients undergoing RYGB:</u></p> <p>↑ baseline 2-HB.</p> <p>After 6 months, ↓ 2-HB, 3-HB, acetic acid, CMPF, and hypoxanthine.</p> <p>After 6 months, ↓ BCAAs; significant correlation between improved HOMA-IR and ↓ valine and BCAAs.</p>            |
|                               |                            |      | Patients with obesity and T2D undergoing RYGB (n=11)           | 41.3 ± 3.0 | 50.5 ± 7.8                         | 8:3   | Decrease of 8.9 ± 1.9                                                |                |                                    |                                                                                                                                                                                                                                                                                                                                                                                                         |

|                    |                            |      |                                                    |                          |                          |      |                          |                        |                                         |                                                                                                                                                                                                                                                     |
|--------------------|----------------------------|------|----------------------------------------------------|--------------------------|--------------------------|------|--------------------------|------------------------|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Tan HC, 2020 [34]  | Prospective cohort study   | SG   | Control group - patients with normal weight (N=10) | Median: 23.2 (22.1–23.7) | Median: 30.3 (25.1–36.1) | 0:10 | NA                       | Fasting plasma samples | Targeted GC-MS and LC-tandem MS         | Outcome: patient with weight loss and improved IR resistance at 6 months after SG (n=8)<br><br><u>Patients undergoing SG:</u><br>After 6 months, ↓ BCAAs, acylcarnitines (C3 and C4) and leucine oxidation.                                         |
|                    |                            |      | Patients with obesity and IR undergoing SG (N=8)   | Median: 38.5 (37.0–40.4) | Median: 29.5 (26.8–41.8) | 5:3  | Median: 30.5 (29.1–32.6) |                        |                                         |                                                                                                                                                                                                                                                     |
| *Yu H, 2015 [59]   | Retrospective cohort study | RYGB | Patients with obesity <sup>§</sup> and T2D (N=38)  | 32.8 ± 3.9               | 43.7 ± 12.8              | 1:1  | 24.9 ± 2.9               | Fasting serum samples  | Targeted UPLC                           | Outcomes: Patients with (n=26) or without (n=12) T2D remission at 2 years<br><br><u>Patients with TD2 remission:</u><br>↑ baseline CDCA relative to the total BAs.<br><br><u>Patients with no TD2 remission:</u><br>1 year after RYGB, ↓ total BAs. |
|                    |                            |      |                                                    | 31.0 ± 3.5               |                          | 7:5  | 24.9 ± 2.5               |                        |                                         |                                                                                                                                                                                                                                                     |
| *Zhao L, 2017 [60] | Prospective cohort study   | RYGB | Patients with obesity <sup>§</sup> and T2D (N=38)  | 32.8 ± 3.9               | 43.7 ± 12.8              | 1:1  | 24.4 ± 2.8               | Fasting serum samples  | Targeted UPLC-QTOFMS (free fatty acids) | Outcomes: Patients with (n=26) or without (n=12) T2D remission at 2 years<br><br><u>Patients with T2D remission:</u><br>↑ baseline ratios of stearic/palmitic acid (C18:0/C16:0) and eicosadienoic/linoleic acid (C20:2 n6/C18:2 n6).               |
|                    |                            |      |                                                    | 31.0 ± 3.5               |                          | 7:5  | 24.8 ± 2.4               |                        |                                         |                                                                                                                                                                                                                                                     |

\* - Both studies had the same participants; § - Asian cohort. Anthropometric characteristics of patients with good and poor outcomes are presented in green or red, respectively. Abbreviations: 2-HB - 2-hydroxybutyrate; 3-HAA - 3-hydroxyanthranilic acid; 3-HB - 3-Hydroxybutyrate; 5-HIAA - 5-hydroxyindoleacetic acid; 5-HTp - 5-hydroxytryptophan; AAA - Aromatic amino acid; AA - Amino acid; BA - Bile acid; BCAA - Branched chain amino acids; BMI - Body mass index; BPD - Biliopancreatic diversion; CDCA - Chenodeoxycholic acid; CE - Cholesterol ester; CMPF - 3- carboxy-4-methyl-5-propyl-2-furanpropionic acid; DCA - Deoxycholic acid; DG - Diglyceride; DJB - Duodenal-jejunal bypass; FFA - Free fatty acids; G - Glycine amidated; GC - Gas chromatography; H-NMR - Proton nuclear magnetic resonance; HCA - Hyocholic acid; HRMS - High resolution mass spectrometry; IR - Insuline Resistance; KynP - Kynurenine pathway; L-DOPA - L-dihydroxyphenylalanine; LC- Liquid chromatography; LCFA - Long-chain fatty acid; LDL - Low density lipoprotein; LPC - Lysophosphatidylcholines; LPE - Lysophosphatidylethanolamines; MCA - Beta-muricholic acid; MS - Mass Spectrometry; NGT- Normal glucose tolerance; PC- Phosphatidylcholines; PE - Phosphatidylethanolamines; PL - Phospholipid; RYGB - Roux-en-Y Gastric Bypass; SG – Sleeve gastrectomy; T2D – Type 2 Diabetes; TCA - Tricarboxylic acid; TDGM - Tryptophan-derived gut microbial metabolites; TG - Triglyceride; UPLC - Ultra-Performance Liquid Chromatography; VLDL - Very low density lipoprotein; WL - Weight loss; WLMP - Weight Loss Maintenance Program.