

Supplementary notes for “A combined lifestyle intervention induces a sensitization of the blood transcriptomic response to a nutrient challenge”

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## SI Table 1: Effects of the intervention on parameters of body composition, health and functioning, and diagnostic measurements.

We tested the effects of the intervention in our selection on all parameters that were significantly affected by the intervention among all participants of the study (van de Rest *et al.*, 2016).

| Body Composition                 | Group | n  | Estimate  | Std. Error | Pr(> t ) | padj     |
|----------------------------------|-------|----|-----------|------------|----------|----------|
| BMI (kg/m <sup>2</sup> )         | Both  | 85 | -0.491484 | 0.031611   | 1.91E-26 | 6.30E-25 |
| BMI (kg/m <sup>2</sup> )         | Men   | 44 | -0.44216  | 0.045998   | 2.82E-12 | 2.33E-11 |
| BMI (kg/m <sup>2</sup> )         | Women | 41 | -0.544418 | 0.042122   | 7.28E-16 | 1.20E-14 |
| Weight (kg)                      | Both  | 85 | -0.372314 | 0.023232   | 2.83E-27 | 1.87E-25 |
| Weight (kg)                      | Men   | 44 | -0.365158 | 0.036661   | 9.73E-13 | 1.07E-11 |
| Weight (kg)                      | Women | 41 | -0.379994 | 0.02822    | 1.91E-16 | 4.19E-15 |
| Waist circumference (cm)         | Both  | 85 | -0.527537 | 0.062773   | 9.40E-13 | 1.07E-11 |
| Waist circumference (cm)         | Men   | 44 | -0.478792 | 0.087542   | 2.15E-06 | 9.46E-06 |
| Waist circumference (cm)         | Women | 41 | -0.579848 | 0.090456   | 1.25E-07 | 7.52E-07 |
| Fat free mass (kg <sup>2</sup> ) | Both  | 85 | -0.219552 | 0.032452   | 3.78E-09 | 2.77E-08 |
| Fat free mass (kg <sup>2</sup> ) | Men   | 44 | -0.236645 | 0.053683   | 9.31E-05 | 2.95E-04 |
| Fat free mass (kg <sup>2</sup> ) | Women | 41 | -0.194882 | 0.034862   | 3.38E-06 | 1.40E-05 |
| Body fat (%)                     | Both  | 85 | -0.226337 | 0.033573   | 4.19E-09 | 2.77E-08 |
| Body fat (%)                     | Men   | 44 | -0.222536 | 0.043525   | 1.15E-05 | 4.47E-05 |
| Body fat (%)                     | Women | 41 | -0.230587 | 0.051856   | 9.40E-05 | 2.95E-04 |

| Health and Functioning             | Group | n  | Estimate  | Std. Error | Pr(> t ) | padj     |
|------------------------------------|-------|----|-----------|------------|----------|----------|
| Systolic bp, (mm Hg) <sup>a</sup>  | Both  | 61 | -0.263422 | 0.078973   | 1.46E-03 | 3.58E-03 |
| Systolic bp, (mm Hg) <sup>a</sup>  | Men   | 35 | -0.16209  | 0.107189   | 1.40E-01 | 2.25E-01 |
| Systolic bp, (mm Hg) <sup>a</sup>  | Women | 26 | -0.39983  | 0.113075   | 1.61E-03 | 3.67E-03 |
| Diastolic bp, (mm Hg) <sup>a</sup> | Both  | 61 | -0.353295 | 0.095142   | 4.50E-04 | 1.24E-03 |
| Diastolic bp, (mm Hg) <sup>a</sup> | Men   | 35 | -0.305263 | 0.128178   | 2.30E-02 | 4.46E-02 |
| Diastolic bp, (mm Hg) <sup>a</sup> | Women | 26 | -0.417955 | 0.143525   | 7.45E-03 | 1.54E-02 |
| REE (kcal/day)                     | Both  | 85 | -0.392101 | 0.06718    | 1.57E-07 | 8.63E-07 |
| REE (kcal/day)                     | Men   | 44 | -0.354197 | 0.097443   | 8.75E-04 | 2.22E-03 |
| REE (kcal/day)                     | Women | 41 | -0.408054 | 0.091904   | 8.95E-05 | 2.95E-04 |
| SPPB                               | Both  | 85 | 0.154229  | 0.111779   | 1.71E-01 | 2.57E-01 |
| SPPB                               | Men   | 44 | 0.198628  | 0.161234   | 2.25E-01 | 3.15E-01 |
| SPPB                               | Women | 41 | 0.106581  | 0.155874   | 4.98E-01 | 5.77E-01 |

| Diagnostic Measures               | Group | n  | Estimate  | Std. Error | Pr(> t ) | padj     |
|-----------------------------------|-------|----|-----------|------------|----------|----------|
| Log(Insulin) (mU/L)               | Both  | 85 | -0.043549 | 0.075492   | 5.66E-01 | 6.44E-01 |
| Log(Insulin) (mU/L)               | Men   | 44 | -0.078906 | 0.112407   | 4.86E-01 | 5.73E-01 |
| Log(Insulin) (mU/L)               | Women | 41 | -0.005605 | 0.100822   | 9.56E-01 | 9.56E-01 |
| Cholesterol (mmol/L) <sup>b</sup> | Both  | 72 | -0.341297 | 0.096833   | 7.47E-04 | 1.97E-03 |
| Cholesterol (mmol/L) <sup>b</sup> | Men   | 37 | -0.29807  | 0.140525   | 4.09E-02 | 7.29E-02 |

|   |       |    |           |          |          |          |
|---|-------|----|-----------|----------|----------|----------|
| Cholesterol (mmol/L) <sup>b</sup>           | Women | 35 | -0.386994 | 0.134375 | 6.84E-03 | 1.46E-02 |
| Cholesterol_HDL (mmol/L) <sup>b</sup>       | Both  | 72 | -0.06479  | 0.069006 | 3.51E-01 | 4.52E-01 |
| Cholesterol_HDL (mmol/L) <sup>b</sup>       | Men   | 37 | 0.156036  | 0.082284 | 6.60E-02 | 1.15E-01 |
| Cholesterol_HDL (mmol/L) <sup>b</sup>       | Women | 35 | -0.298234 | 0.098785 | 4.78E-03 | 1.05E-02 |
| LDL_Cholesterol (mmol/L) <sup>b</sup>       | Both  | 72 | -0.342264 | 0.081633 | 7.83E-05 | 2.87E-04 |
| LDL_Cholesterol (mmol/L) <sup>b</sup>       | Men   | 37 | -0.414995 | 0.12158  | 1.60E-03 | 3.67E-03 |
| LDL_Cholesterol (mmol/L) <sup>b</sup>       | Women | 35 | -0.265376 | 0.108364 | 1.96E-02 | 3.93E-02 |
| Log(triglycerides) (mmol/L) <sup>b</sup>    | Both  | 72 | 0.101197  | 0.046541 | 3.30E-02 | 6.23E-02 |
| Log(triglycerides) (mmol/L) <sup>b</sup>    | Men   | 37 | 0.265653  | 0.068206 | 4.09E-04 | 1.20E-03 |
| Log(triglycerides) (mmol/L) <sup>b</sup>    | Women | 35 | -0.072656 | 0.048663 | 1.45E-01 | 2.27E-01 |
| TSH (mU/L) <sup>b</sup>                     | Both  | 72 | -0.092626 | 0.069409 | 1.86E-01 | 2.67E-01 |
| TSH (mU/L) <sup>b</sup>                     | Men   | 37 | -0.12726  | 0.093594 | 1.82E-01 | 2.67E-01 |
| TSH (mU/L) <sup>b</sup>                     | Women | 35 | -0.056013 | 0.103989 | 5.94E-01 | 6.53E-01 |
| DHEAS (nmol/L) <sup>c</sup>                 | Both  | 85 | -0.031264 | 0.034452 | 3.67E-01 | 4.57E-01 |
| DHEAS (nmol/L) <sup>c</sup>                 | Men   | 44 | 0.015244  | 0.046221 | 7.43E-01 | 7.91E-01 |
| DHEAS (nmol/L) <sup>c</sup>                 | Women | 41 | -0.081176 | 0.05082  | 1.18E-01 | 2.00E-01 |
| Log(Leptin) (μg/L)                          | Both  | 85 | -0.373918 | 0.045596 | 2.40E-12 | 2.27E-11 |
| Log(Leptin) (μg/L)                          | Men   | 44 | -0.413556 | 0.069387 | 4.18E-07 | 2.12E-06 |
| Log(Leptin) (μg/L)                          | Women | 41 | -0.331379 | 0.05839  | 1.35E-06 | 6.37E-06 |
| Log(Adiponectin) (mg/L)                     | Both  | 61 | 0.11647   | 0.05465  | 3.72E-02 | 6.82E-02 |
| Log(Adiponectin) (mg/L)                     | Men   | 35 | 0.263722  | 0.067419 | 4.17E-04 | 1.20E-03 |
| Log(Adiponectin) (mg/L)                     | Women | 26 | -0.081754 | 0.075869 | 2.92E-01 | 3.93E-01 |
| IGF1 (nmol/L)                               | Both  | 85 | 0.024569  | 0.05319  | 6.45E-01 | 6.98E-01 |
| IGF1 (nmol/L)                               | Men   | 44 | 0.057414  | 0.072316 | 4.32E-01 | 5.28E-01 |
| IGF1 (nmol/L)                               | Women | 41 | -0.01068  | 0.078894 | 8.93E-01 | 9.07E-01 |
| IGFBP-3 (mg/L)                              | Both  | 85 | -0.079955 | 0.08051  | 3.24E-01 | 4.27E-01 |
| IGFBP-3 (mg/L)                              | Men   | 44 | -0.176558 | 0.119957 | 1.48E-01 | 2.28E-01 |
| IGFBP-3 (mg/L)                              | Women | 41 | 0.023716  | 0.105355 | 8.23E-01 | 8.49E-01 |
| IGF-1:IGFBP-3                               | Both  | 85 | 0.099248  | 0.084379 | 2.43E-01 | 3.34E-01 |
| IGF-1:IGFBP-4                               | Men   | 44 | 0.21594   | 0.13713  | 1.23E-01 | 2.02E-01 |
| IGF-1:IGFBP-5                               | Women | 41 | -0.025982 | 0.092444 | 7.80E-01 | 8.17E-01 |
| CRP (high-sensitivity), (mg/L) <sup>a</sup> | Both  | 85 | -0.103727 | 0.1118   | 3.56E-01 | 4.52E-01 |
| CRP (high-sensitivity), (mg/L) <sup>a</sup> | Men   | 44 | -0.085597 | 0.156706 | 5.88E-01 | 6.53E-01 |
| CRP (high-sensitivity), (mg/L) <sup>a</sup> | Women | 41 | -0.123183 | 0.161458 | 4.50E-01 | 5.40E-01 |
| ft3 (pmol/L)                                | All   | 85 | -0.153412 | 0.034946 | 3.28E-05 | 8.20E-05 |
| ft3 (pmol/L)                                | Men   | 44 | -0.155909 | 0.045026 | 1.22E-03 | 2.12E-03 |
| ft3 (pmol/L)                                | Women | 41 | -0.150732 | 0.054567 | 8.63E-03 | 1.14E-02 |



<sup>a</sup>Individuals on antihypertensive medication were excluded from this analysis

<sup>b</sup>Individuals on lipid-lowering medication were excluded from this analysis

## SI Table 2: Differentially expressed genes

See SI\_Table\_2.xlsx for a list (and summary) of all differentially expressed genes

### SI Table 3: Functional Enrichments in different sets

Supplementary file SI\_Table\_3.xlsx provides the significant terms identified in:

- The set of down-regulated genes in response to the nutrient challenge, before or after the intervention, in males or females
- The set of up-regulated genes in response to the nutrient challenge, before or after the intervention, in males or females
- The set of up or down regulated genes in response to the intervention in a fasted state
- The set of up or down regulated genes in response to the intervention in a postprandial state.
- The set of genes found to affect the trajectory of the nutrient challenge response in males
- The set of genes found to affect the trajectory of the nutrient challenge response in females
- The sets of genes in each module identified in the male correlation network
- The sets of genes in each module identified in the female correlation network

## SI Table 4: Blood cell type counts

No blood cell type was differently represented between at baseline versus after the intervention ( $p > 0.05$ ), also not in a sex-stratified analysis. Blood cell type count percentages were assessed with a linear mixed model with  $percentage \sim intervention + sex + (1|person)$  for the bulk analysis, and with  $\sim intervention + (1|person\_id)$  for the sex-stratified analysis.

| Estimate  | Std..Error | t.value   | p-value  | Celltype     | Condition | q-value  |
|-----------|------------|-----------|----------|--------------|-----------|----------|
| 0.001534  | 0.001324   | 1.158072  | 2.49E-01 | hematocrit   | All       | 5.27E-01 |
| 0.008282  | 0.104856   | 0.078987  | 9.37E-01 | eosinophiles | All       | 9.54E-01 |
| 0.031288  | 0.022545   | 1.387845  | 1.67E-01 | basophiles   | All       | 4.27E-01 |
| -0.028896 | 0.497674   | -0.058062 | 9.54E-01 | neutrophiles | All       | 9.54E-01 |
| -0.168896 | 0.427137   | -0.395414 | 6.93E-01 | lymphocyte   | All       | 8.66E-01 |
| 0.163681  | 0.167841   | 0.975214  | 3.31E-01 | monocytes    | All       | 5.84E-01 |
| -0.000854 | 0.001895   | -0.450587 | 6.53E-01 | hematocrit   | Male      | 8.52E-01 |
| 0.097683  | 0.150023   | 0.651121  | 5.17E-01 | eosinophiles | Male      | 8.16E-01 |
| 0.056951  | 0.037235   | 1.529527  | 1.30E-01 | basophiles   | Male      | 3.90E-01 |
| 0.345122  | 0.697494   | 0.494803  | 6.22E-01 | neutrophiles | Male      | 8.48E-01 |
| -0.835732 | 0.604836   | -1.38175  | 1.71E-01 | lymphocyte   | Male      | 4.27E-01 |
| 0.342683  | 0.215171   | 1.592607  | 1.15E-01 | monocytes    | Male      | 3.84E-01 |
| 0.003951  | 0.001823   | 2.167065  | 3.32E-02 | hematocrit   | Female    | 1.73E-01 |
| -0.082222 | 0.146733   | -0.560353 | 5.77E-01 | eosinophiles | Female    | 8.24E-01 |
| 0.005309  | 0.025166   | 0.210949  | 8.33E-01 | basophiles   | Female    | 9.54E-01 |
| -0.407531 | 0.712088   | -0.572304 | 5.69E-01 | neutrophiles | Female    | 8.24E-01 |
| 0.506173  | 0.597637   | 0.846957  | 4.00E-01 | lymphocyte   | Female    | 6.66E-01 |
| -0.017531 | 0.257921   | -0.06797  | 9.46E-01 | monocytes    | Female    | 9.54E-01 |

## SI Table 5: Functional enrichment of modules identified in participant cluster gene correlation networks

See SI\_Table\_5.xlsx for a list (and summary) of all functional enrichments per detected module per participant cluster.

SI Table 6: Comparison between the HuMet Standard Liquid Diet and the GOTO nutridrink

Caseine AA concentrations were taken from (Bjørndal *et al.*, 2015).

| <b>Component</b>              | <b>GOTO Nutricia<br/>energy drink,<br/>vanilla</b> | <b>Humet Fresubin<br/>Energy Drink,<br/>chocolate</b> |
|-------------------------------|--|---|
| Carbohydrates                 | 18.4   | 18.8  |
| glucose                       | 0.1  | 0.2   |
| fructose                      | 0  | 0   |
| maltose                       | 0.6  | 0.33  |
| sucrose                       | 6  | 5.5   |
| lactose                       | 0.025  | 0.27  |
| polysacc                      | 11.3   | 12.45   |
|                               |  |   |
|                               |  |   |
| Protein*                      | 5.9  | 6   |
| lysine                        | 0.4366   | 0.45  |
| threonine                     | 0.2242   | 0.25  |
| methionine                    | 0.1534   | 0.15  |
| phenylalanine                 | 0.295  | 0.28  |
| tryptophan                    | 0.0708   | 0.08  |
| valine                        | 0.3363   | 0.39  |
| leucine                       | 0.5192   | 0.54  |
| isoleucine                    | 0.2832   | 0.3   |
| tyrosone                      | 0.3127   | 0.3   |
| cysteine                      | 0.0236   | 0.004   |
| histidine                     | 0.1534   | 0.15  |
| arginine                      | 0.2124   | 0.21  |
| glutamine                     |  | 0.47  |
| glycine                       | 0.1062   | 0.11  |
| alanine                       | 0.1534   | 0.18  |
| proline                       | 0.6903   | 0.54  |
| serine                        | 0.3186   | 0.32  |
| glutamic acid                 | 1.2272   | 0.71  |
| aspartic acid &<br>asparagine | 0.3835   | 0.57  |

SI Table 7: Metabolites tested

| Abbreviation | Name  | Group           | Subgroup                   | Unit   |
|--------------|---|-----------------|----------------------------|--------|
| Ala          | Alanine   | Amino acids     | Amino acids                | mmol/l |
| Gln          | Glutamine   | Amino acids     | Amino acids                | mmol/l |
| His          | Histidine   | Amino acids     | Amino acids                | mmol/l |
| Phe          | Phenylalanine   | Amino acids     | Aromatic amino acids       | mmol/l |
| Tyr          | Tyrosine  | Amino acids     | Aromatic amino acids       | mmol/l |
| Ile          | Isoleucine  | Amino acids     | Branched-chain amino acids | mmol/l |
| Leu          | Leucine   | Amino acids     | Branched-chain amino acids | mmol/l |
| Val          | Valine  | Amino acids     | Branched-chain amino acids | mmol/l |
| ApoA1        | Apolipoprotein A-I  | Apolipoproteins | Apolipoproteins            | g/l    |
| ApoB         | Apolipoprotein B  | Apolipoproteins | Apolipoproteins            | g/l    |
| Total-C      | Total cholesterol   | Cholesterol     | Cholesterol                | mmol/l |
| LDL-C        | Total cholesterol in 3 LDL subclasses                     | Cholesterol     | Cholesterol                | mmol/l |
| HDL-C        | Total cholesterol in HDL                                  | Cholesterol     | Cholesterol                | mmol/l |
| HDL2-C       | Total cholesterol in HDL2                                 | Cholesterol     | Cholesterol                | mmol/l |
| HDL3-C       | Total cholesterol in HDL3                                 | Cholesterol     | Cholesterol                | mmol/l |
| VLDL-C       | Total cholesterol in VLDL                                 | Cholesterol     | Cholesterol                | mmol/l |
| MUFA %       | Ratio of monounsaturated fatty acids to total fatty acids | Fatty acids     | Fatty acid ratios          | %      |
| Omega-3 %    | Ratio of omega-3 fatty acids to total fatty acids         | Fatty acids     | Fatty acid ratios          | %      |
| Omega-6 %    | Ratio of omega-6 fatty acids to total fatty acids         | Fatty acids     | Fatty acid ratios          | %      |
| PUFA %       | Ratio of polyunsaturated fatty acids to total ...         | Fatty acids     | Fatty acid ratios          | %      |
| SFA %        | Ratio of saturated fatty acids to total fatty ...         | Fatty acids     | Fatty acid ratios          | %      |
| Unsaturation | Degree of unsaturation                                    | Fatty acids     | Fatty acids                | degree |
| DHA          | Docosahexaenoic acid; 22:6                                | Fatty acids     | Fatty acids                | mmol/l |
| LA           | Linoleic acid; 18:2                                       | Fatty acids     | Fatty acids                | mmol/l |

|                |   |                                |                                       |             |
|----------------|---|--------------------------------|---------------------------------------|-------------|
| MUFA           | Monounsaturated fatty acids; 16:1, 18:1           | Fatty acids                    | Fatty acids                           | mmol/l      |
| Omega-3        | Omega-3 fatty acids                               | Fatty acids                    | Fatty acids                           | mmol/l      |
| Omega-6        | Omega-6 fatty acids                               | Fatty acids                    | Fatty acids                           | mmol/l      |
| PUFA           | Polyunsaturated fatty acids                       | Fatty acids                    | Fatty acids                           | mmol/l      |
| SFA            | Saturated fatty acids                             | Fatty acids                    | Fatty acids                           | mmol/l      |
| Total-FA       | Total fatty acids                                 | Fatty acids                    | Fatty acids                           | mmol/l      |
| Albumin        | Albumin   | Fluid balance                  | Fluid balance                         | signal area |
| Creatinine     | Creatinine  | Fluid balance                  | Fluid balance                         | mmol/l      |
| Phosphatidylc  | Phosphatidylcholine                               | Glycerides and phospholipids   | Glycerides and phospholipids          | mmol/l      |
| Sphingomyelins | Sphingomyelins                                    | Glycerides and phospholipids   | Glycerides and phospholipids          | mmol/l      |
| Cholines       | Total cholines                                    | Glycerides and phospholipids   | Glycerides and phospholipids          | mmol/l      |
| Total-TG       | Total triglycerides                               | Glycerides and phospholipids   | Glycerides and phospholipids          | mmol/l      |
| Phosphoglyc    | Total phosphoglycerides                           | Glycerides and phospholipids   | Glycerides and phospholipids          | mmol/l      |
| Citrate        | Citrate   | Glycolysis related metabolites | Glycolysis related metabolites        | mmol/l      |
| Glucose        | Glucose   | Glycolysis related metabolites | Glycolysis related metabolites        | mmol/l      |
| Lactate        | Lactate   | Glycolysis related metabolites | Glycolysis related metabolites        | mmol/l      |
| Pyruvate       | Pyruvate  | Glycolysis related metabolites | Glycolysis related metabolites        | mmol/l      |
| GlycA          | Glycoprotein acetyls (GlycA)                      | Inflammation                   | Inflammation                          | mmol/l      |
| bOHbutyrate    | 3-hydroxybutyrate                                 | Ketone bodies                  | Ketone bodies                         | mmol/l      |
| Acetate        | Acetate   | Ketone bodies                  | Ketone bodies                         | mmol/l      |
| Acetoacetate   | Acetoacetate                                      | Ketone bodies                  | Ketone bodies                         | mmol/l      |
| HDL size       | Mean diameter for HDL particles                   | Lipoprotein particle sizes     | Lipoprotein particle sizes            | nm          |
| LDL size       | Mean diameter for LDL particles                   | Lipoprotein particle sizes     | Lipoprotein particle sizes            | nm          |
| VLDL size      | Mean diameter for VLDL particles                  | Lipoprotein particle sizes     | Lipoprotein particle sizes            | nm          |
| XXL-VLDL-L     | Total lipids in chylomicrons and extremely lar... | Lipoprotein subclasses         | Chylomicrons and extremely large VLDL | mmol/l      |

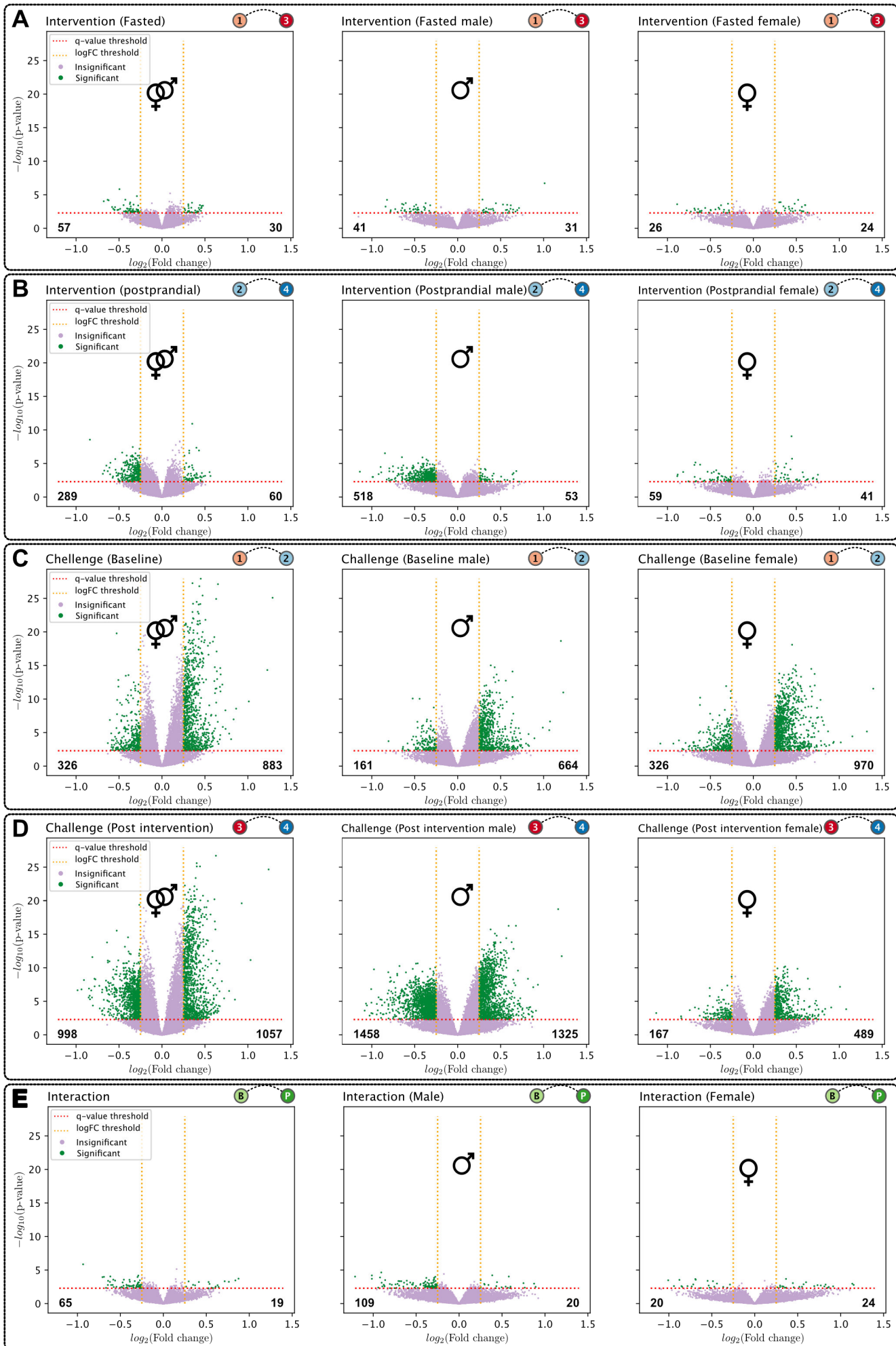


|           |                                 |                        |                 |        |
|-----------|---------------------------------|------------------------|-----------------|--------|
| IDL-C     | Total cholesterol in IDL        | Lipoprotein subclasses | IDL             | mmol/l |
| IDL-L     | Total lipids in IDL             | Lipoprotein subclasses | IDL             | mmol/l |
| L-HDL-L   | Total lipids in large HDL       | Lipoprotein subclasses | Large HDL       | mmol/l |
| L-LDL-L   | Total lipids in large LDL       | Lipoprotein subclasses | Large LDL       | mmol/l |
| L-VLDL-L  | Total lipids in large VLDL      | Lipoprotein subclasses | Large VLDL      | mmol/l |
| M-HDL-L   | Total lipids in medium HDL      | Lipoprotein subclasses | Medium HDL      | mmol/l |
| M-LDL-L   | Total lipids in medium LDL      | Lipoprotein subclasses | Medium LDL      | mmol/l |
| M-VLDL-L  | Total lipids in medium VLDL     | Lipoprotein subclasses | Medium VLDL     | mmol/l |
| S-HDL-L   | Total lipids in small HDL       | Lipoprotein subclasses | Small HDL       | mmol/l |
| S-LDL-L   | Total lipids in small LDL       | Lipoprotein subclasses | Small LDL       | mmol/l |
| S-VLDL-L  | Total lipids in small VLDL      | Lipoprotein subclasses | Small VLDL      | mmol/l |
| XL-HDL-L  | Total lipids in very large HDL  | Lipoprotein subclasses | Very large HDL  | mmol/l |
| XL-VLDL-L | Total lipids in very large VLDL | Lipoprotein subclasses | Very large VLDL | mmol/l |
| XS-VLDL-L | Total lipids in very small VLDL | Lipoprotein subclasses | Very small VLDL | mmol/l |

## SI Figure 1: Volcanoplots for combined and sex-stratified tests

Volcano plots for each differential expression test. Red lines indicate the FDR adjusted p-value threshold. Yellow lines indicate effect size thresholds. Green points represent significant genes, and purple points are not significant. In each panel, we provide the unstratified, as well as the sex stratified results. The number of genes differentially expressed are indicated in bold text.

- A) Fasted intervention effect
- B) Postprandial intervention effect. Note that interpreting the intervention effect in only the postprandial samples is problematic, as it ignores the context of the fasted starting point in terms of investigating the response. A proper investigation of this effect can be seen in the interaction test (given below).
- C) Baseline challenge effect
- D) Post-intervention challenge effect
- E) Intervention\*challenge Interaction effect

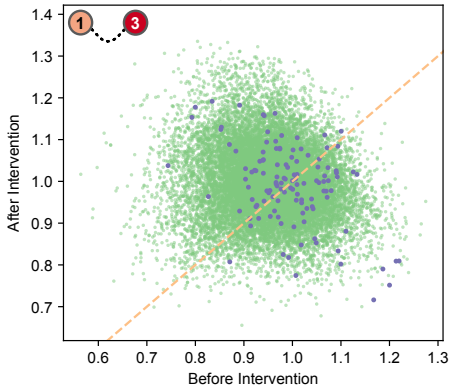


## SI Figure 2: Variation in participant gene expression before vs. after the intervention, per gene

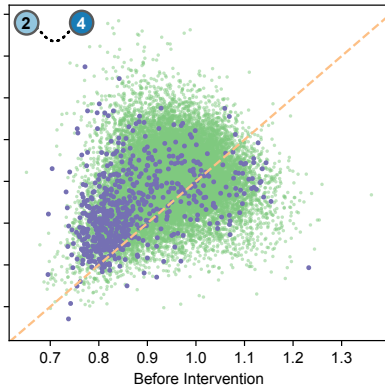
In each panel, we provide the gene variation across samples before (x-axis) vs after the intervention (y-axis). Each point is a gene, and purple points indicate genes that were significantly affected by the intervention. For panels from left to right:

1. For fasted samples, i.e. sample 1 vs sample 3
2. For postprandial samples, i.e. sample 2 vs sample 4
3. For the nutrient challenge effect, i.e. sample (2/1) vs sample (4/3)

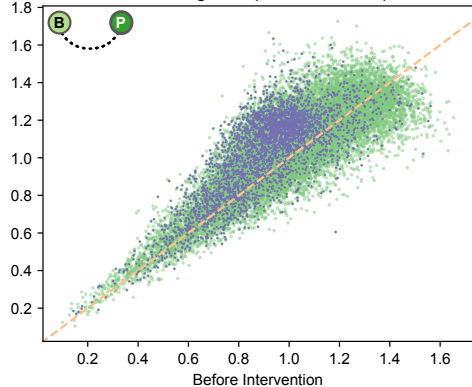
Fasted gene expression



Postprandial gene expression



Nutrient Challenge Response Gene Expression

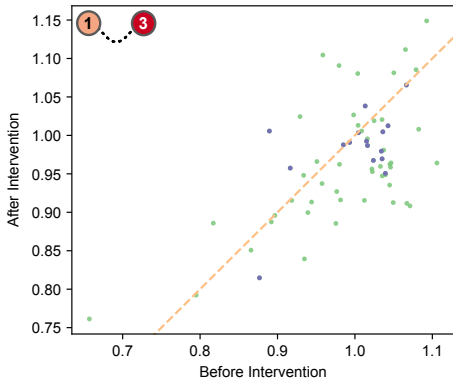


### SI Figure 3: Variation in participant metabolite abundance before vs. after the intervention, per metabolite

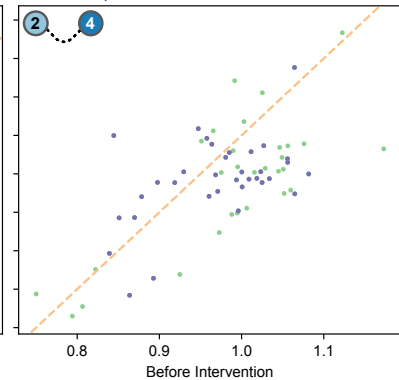
In each panel, we provide the metabolite variation across samples before (x-axis) vs after the intervention (y-axis). Each point is a metabolite, and purple points indicate metabolites that were significantly affected by the intervention. For panels from left to right:

1. For fasted samples, i.e. sample 1 vs sample 3
2. For postprandial samples, i.e. sample 2 vs sample 4
3. For the nutrient challenge effect, i.e. sample (2/1) vs sample (4/3)

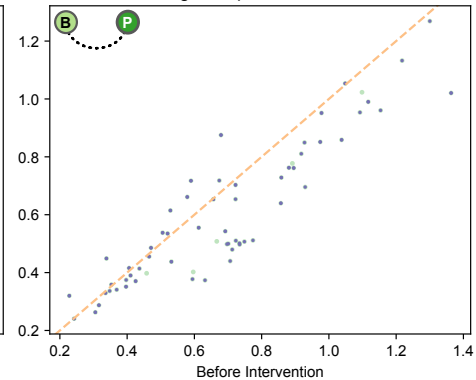
Fasted Metabolite Abundance



Postprandial Metabolite Abundance



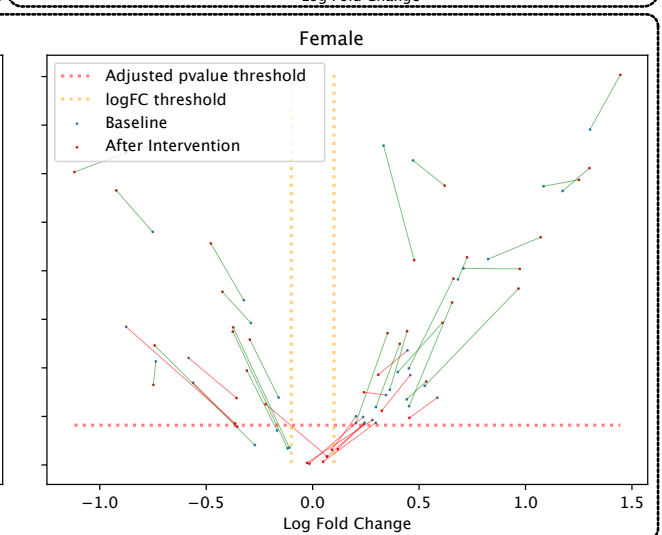
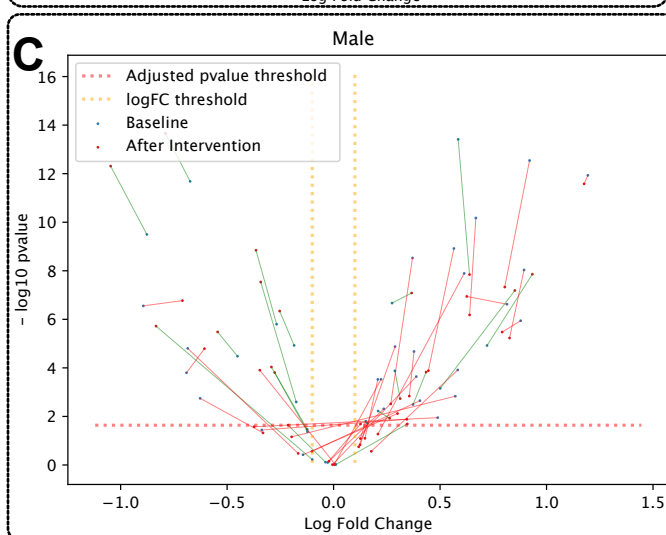
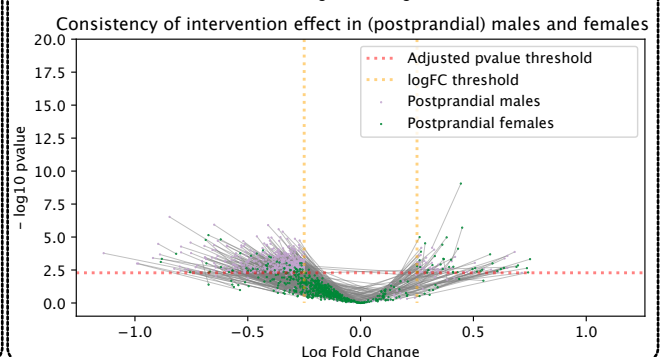
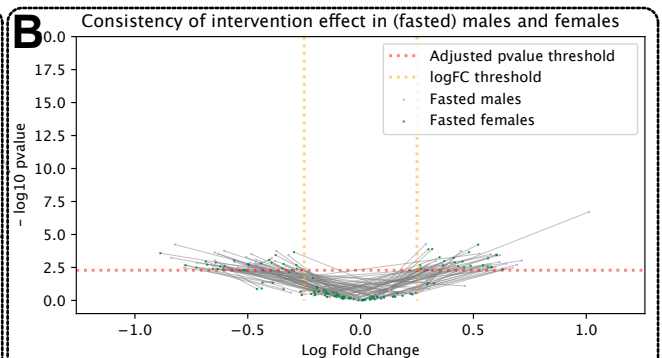
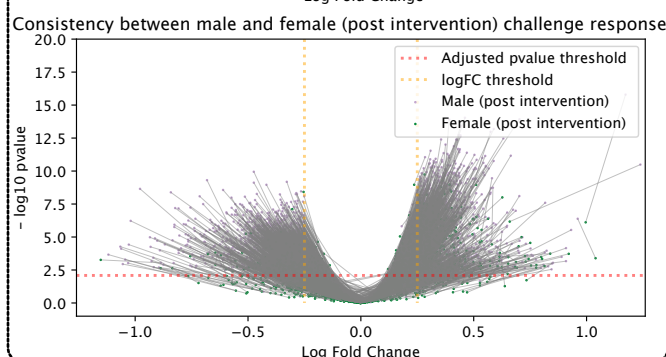
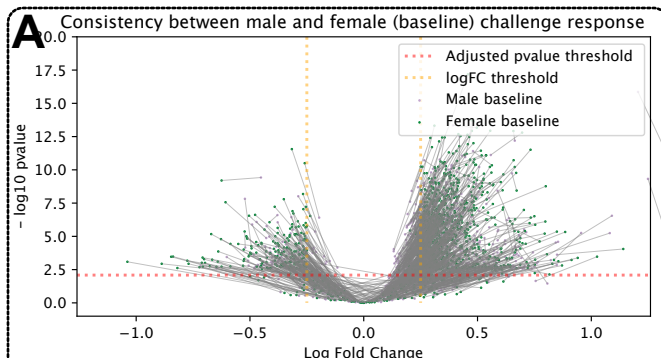
Nutrient Challenge Response Metabolite Abundance



## SI Figure 4: Consistency of responses

- A) Consistency of responses between males and females in the transcriptomic nutrient challenge response. Provided as a paired-volcano plot. Green points are female gene differential expression test results, linked by a line to the male response in purple. Above, the baseline response, below, the post-intervention response.
- B) Consistency of responses between males and females in the transcriptomic intervention response. Provided as a paired-volcano plot. Green points are female gene differential expression test results, linked by a line to the male response in purple. Above, the fasted response, below, the postprandial response.
- C) Consistency of the metabolomic nutrient response. Provided as a paired-volcano plot. Left panel is for males, and right panel is for females. Blue points are baseline metabolite responses, red points, connected to blue points with a line are post intervention metabolomic responses.





## SI Figure 5: Stratified Gene expression levels

Expression levels shown in Figure 3 are for both males and females together. Here we provide the same plots as in Figure 3, but stratified for males and females, to show that the effects are consistent across the sexes, and differ in intensity. Each pair of points represents an individual's response to the nutrient challenge, on the left at baseline, and on the right following the intervention. Each pair is connected with an orange line if the expression is increased, and with a blue line if the expression is decreased. In SI Figure 3, we provide these figures separately for males and females.

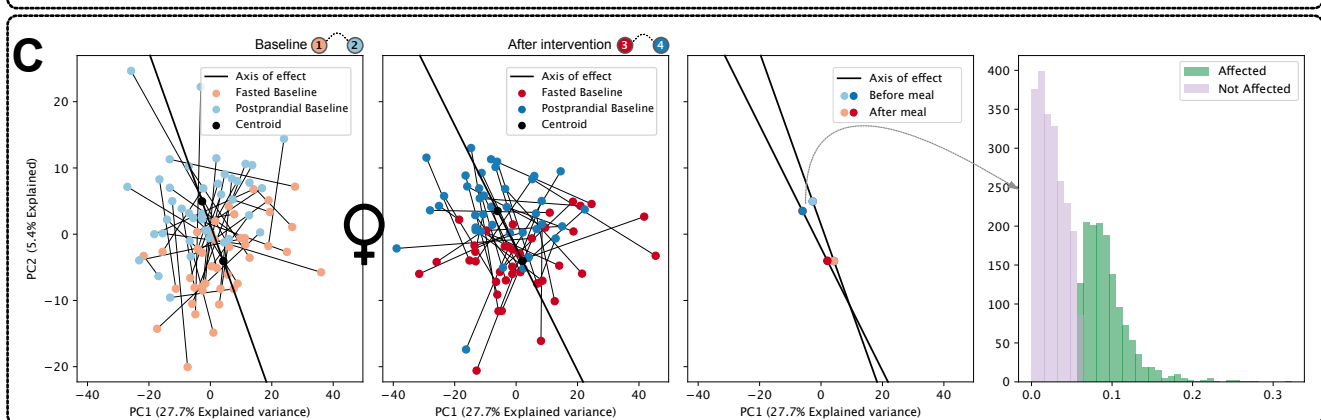
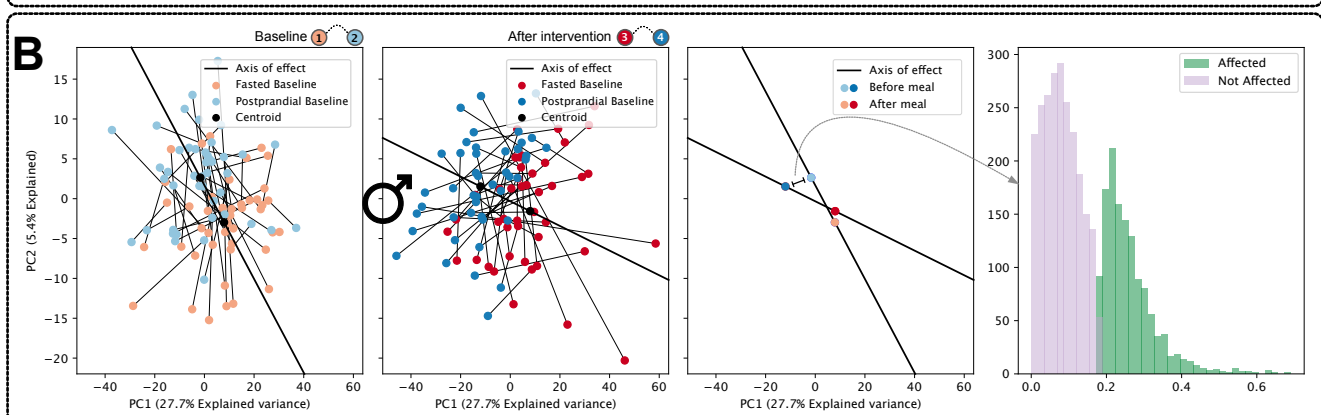
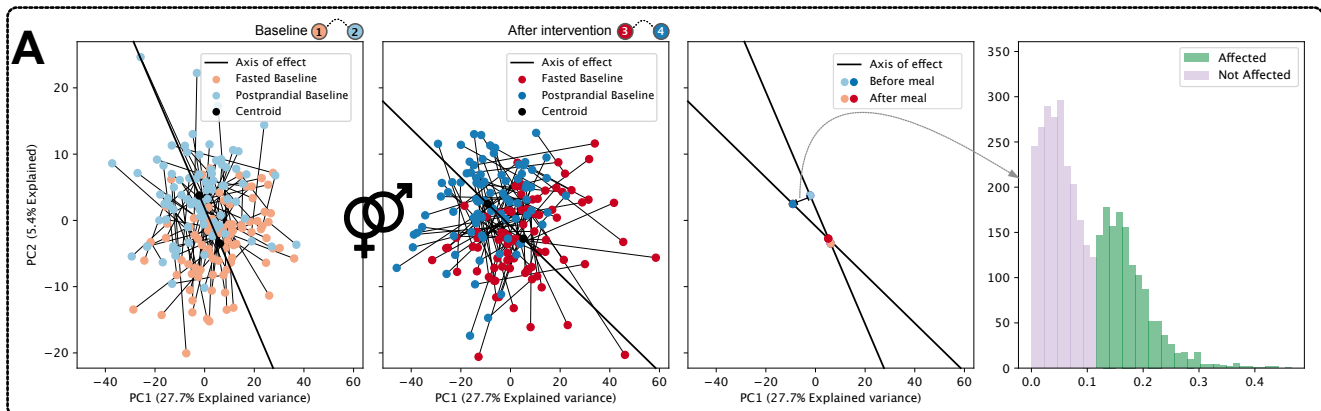
- A)** The translational genes
- B)** The innate immune genes, and
- C)** The stress genes.



## SI Figure 6: Individual trajectories in PC Space

PC Trajectories of individuals in the study. In the first panel, the trajectories for individuals in response to the baseline nutrient challenge are displayed. Red points indicate fasted transcriptomic states, and blue points the postprandial states, projected in PC space. States from the same individual are connected with a line. The average trajectory between the two states is indicated with a thick black line. In the second panel, the same trajectories are given, but after the intervention. The third panel superimposes the baseline and post-intervention trajectories. The final panel indicates the gene expression level differences between the postprandial states (Materials and Methods).

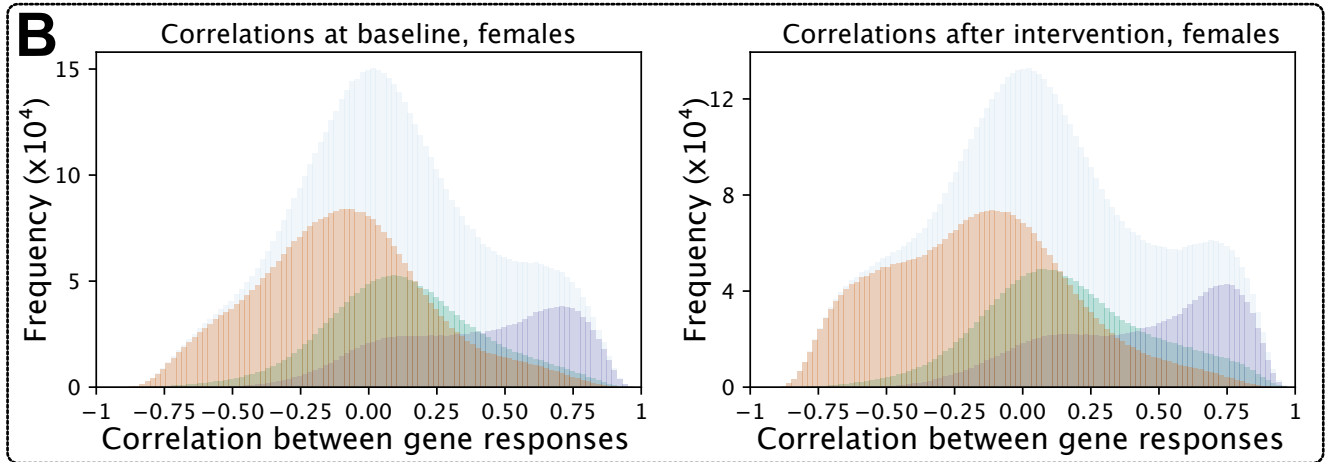
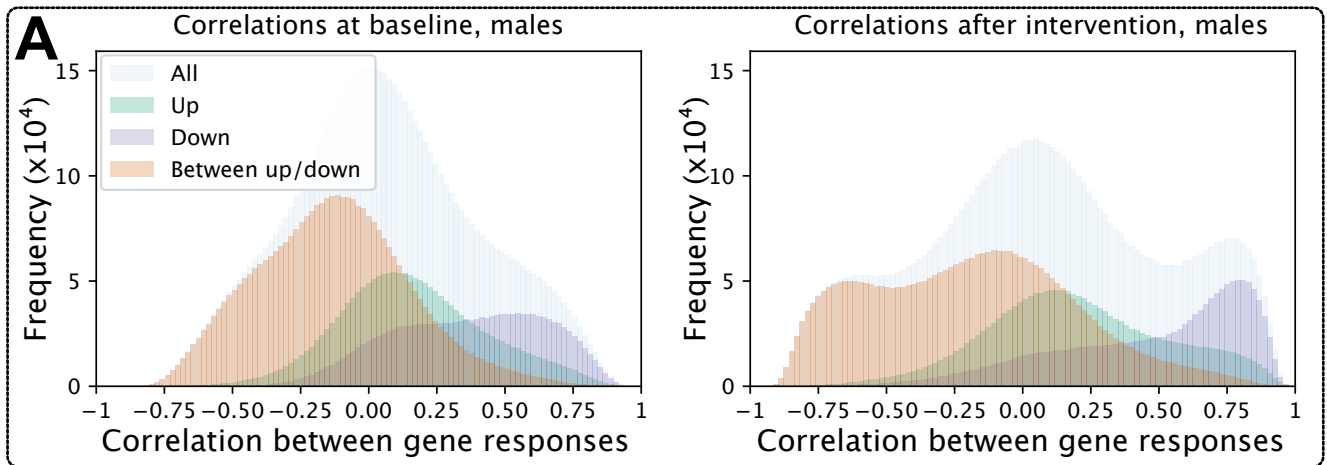
- A) For all participants
- B) For males only
- C) For females only



## SI Figure 7: Change in correlation structure in males and females

For simplicity, Figure 5A showed the changes in correlation distributions for only male samples. Here we provide the same figures for males and females. The distribution of pairwise postprandial gene-response correlations at baseline (left) and post-intervention (right). The green peak represents all correlations between up-regulated genes, the blue peak between all down-regulated genes, and the brown peak between up- and down-regulated genes.

- A) For males only
- B) For females only

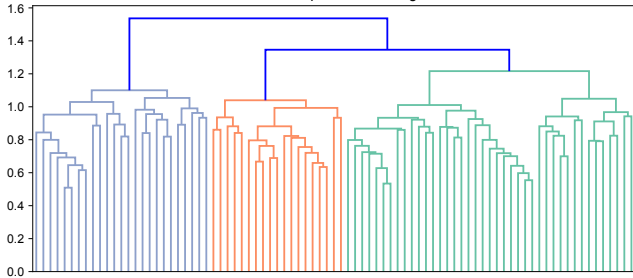


## SI Figure 8: Participant clustering

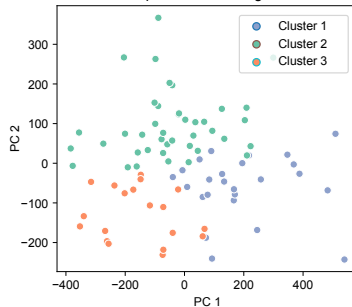
- A) Hierarchical clustering of participant gene nutrient intervention responses into three clusters (see Materials and Methods)
- B) Clustering determined in A) projected onto a PCA reduction of the participant gene nutrient intervention response matrix. Same colours as in A). Each point is a participant.
- C) The same PCA as in B, but with the colours indicating sex.



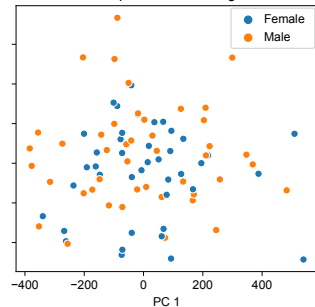
Gene response clustering



Gene response embedding - Clusters



Gene response embedding - Sex



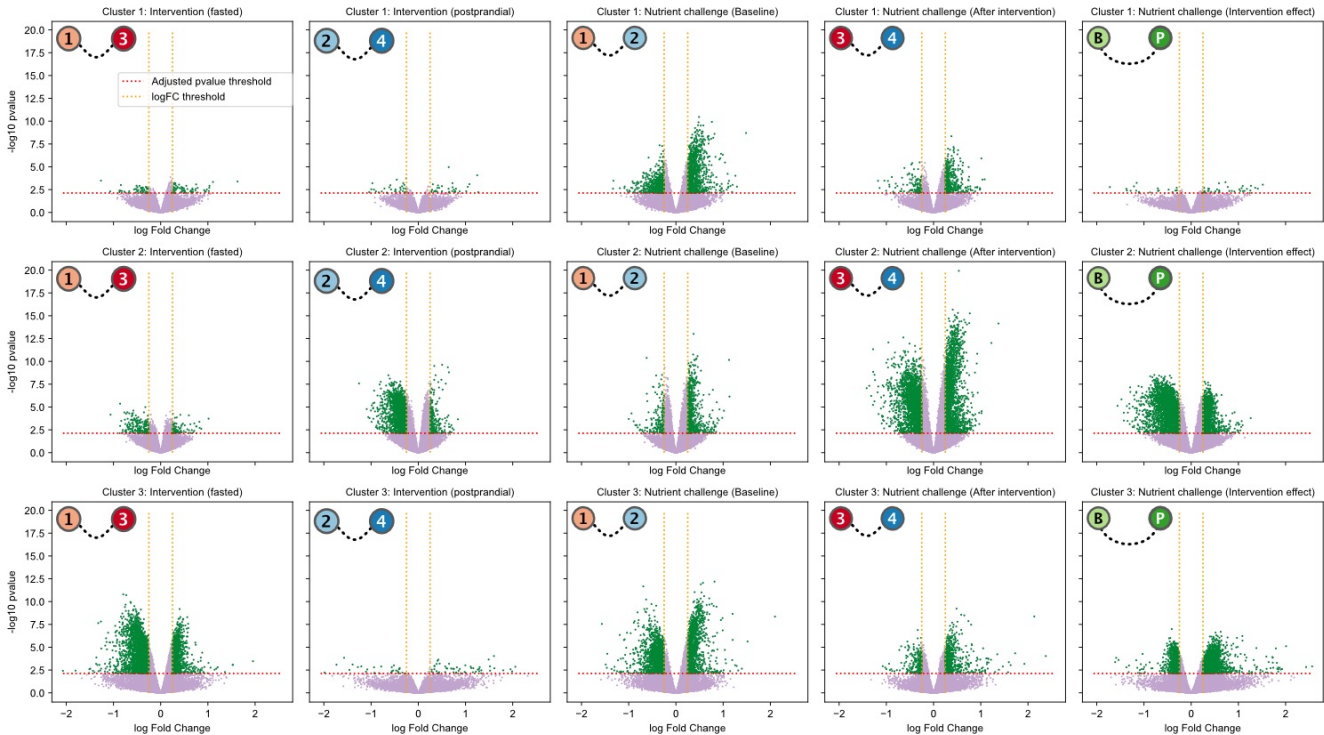
## SI Figure 9: Differential gene expression per participant cluster

Volcano plots for each differential expression test. Red lines indicate the FDR adjusted p-value threshold. Yellow lines indicate effect size thresholds. Green points represent significant genes, and purple points are not significant.

- Top row: Cluster 1
- Middle row: Cluster 2
- Bottom row: Cluster 3.

For each row, we provide the results for the:

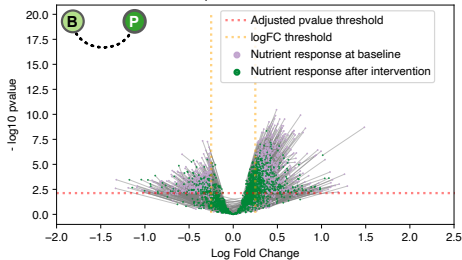
1. Fasted intervention effect
2. Postprandial intervention effect. Note that interpreting the intervention effect in only the postprandial samples is problematic, as it ignores the context of the fasted starting point in terms of investigating the response. A proper investigation of this effect can be seen in the interaction test (given below).
3. Baseline challenge effect
4. Post-intervention challenge effect
5. Intervention\*challenge Interaction effect



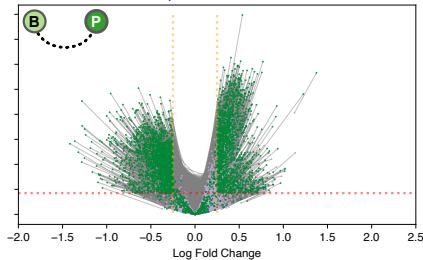
### SI Figure 10: Paired volcano plot per participant cluster

Paired-volcano-plots with results for each participant cluster only. Each pair of points, connected by a line, represent the same gene at two conditions, the postprandial response, in purple at baseline, and in green following the intervention. Only genes which were significant in either of the two conditions are shown.

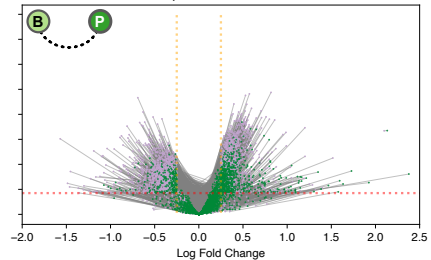
Cluster 1: Nutrient response before vs after intervention



Cluster 2: Nutrient response before vs after intervention



Cluster 3: Nutrient response before vs after intervention

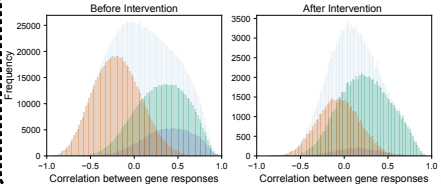


## SI Figure 11: Gene correlation structure per participant cluster

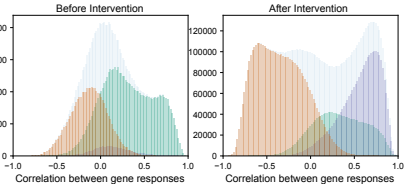
Modulation of the correlation network as a result of the intervention in each participant cluster. Within each cluster, we provide:

- Left panel: The distribution of pairwise postprandial gene-response correlations at baseline. The green peak represents all correlations between up-regulated genes, the blue peak between all down-regulated genes, and the brown peak between up- and down-regulated genes.
- Right panel: The distribution of pairwise postprandial gene-response correlations following the intervention.

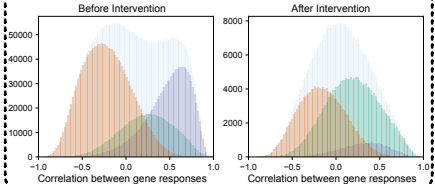
Cluster 1



Cluster 2



Cluster 3



## SI Figure 12: Modules in gene correlation networks per participant cluster, and their enrichments.

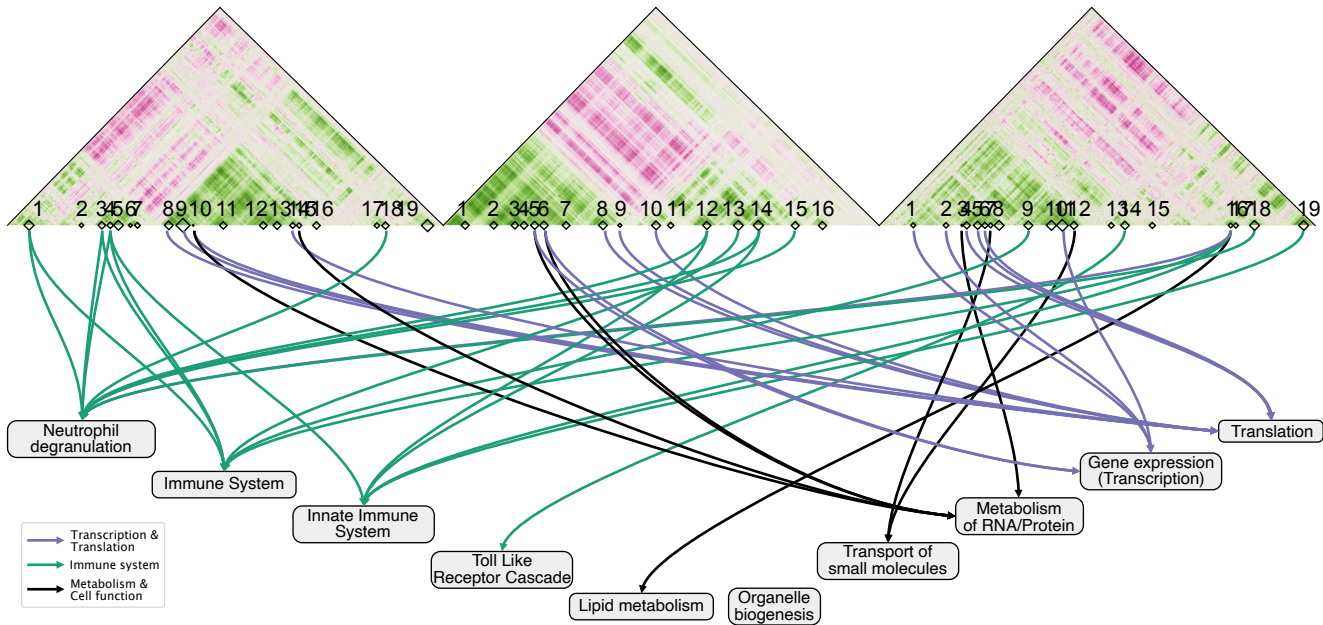
The correlation network between intervention-postprandial gene-responses. Green indicates a higher positive correlation, and red a higher negative correlation. The identified submodules are indicated, and arrows indicate the top functional enrichments for those clusters. Each network represents the gene nutrient intervention response per participant cluster.



Cluster 1

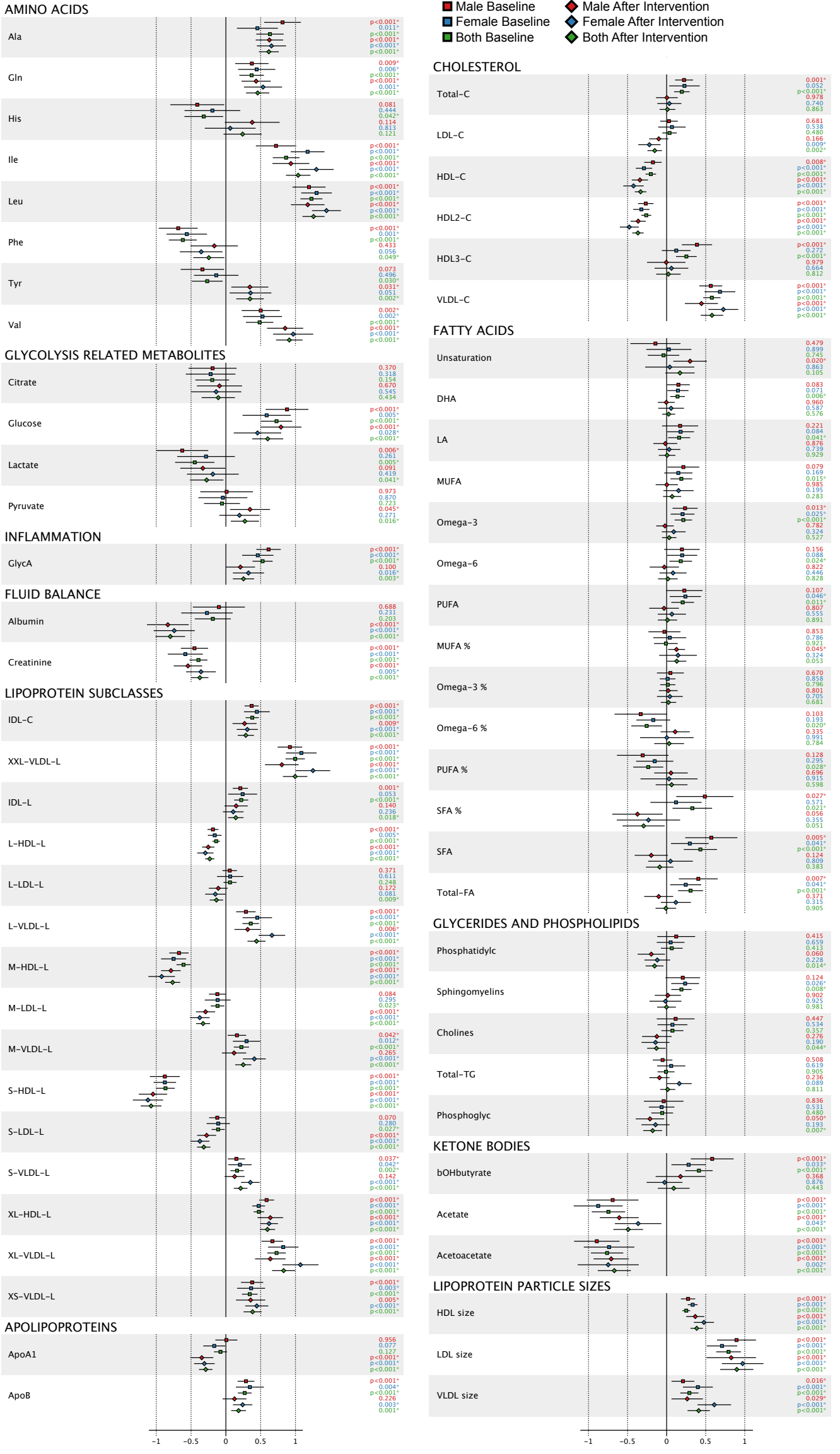
Cluster 2

Cluster 3



### SI Figure 13: Changes in metabolite levels by nutrient challenge

Changes in metabolite levels due to the nutrient challenge were investigated at baseline and after the intervention in men, women, and in bulk. The forest plot shows the results of the analysis described in the materials and methods.



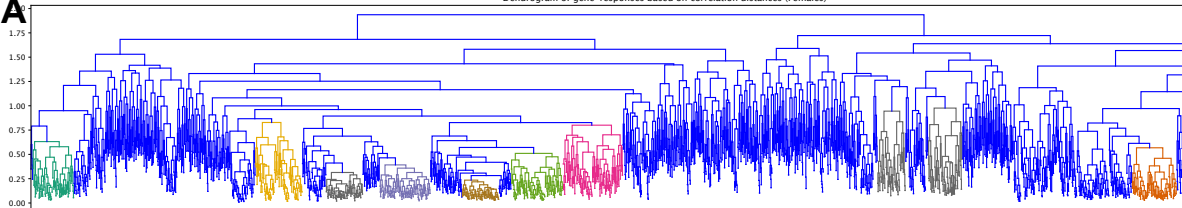
## SI Figure 14: Modules detected in differentially expressed genes

Hierarchical clustering of differentially expressed gene responses to nutrient intake. The height of the tree refers to the 1-p correlation between genes. (i.e. a height of 0 indicates a correlation of 1, 1 indicates 0 correlation, 2 indicates -1 correlation). Different clusters are indicated in colors.

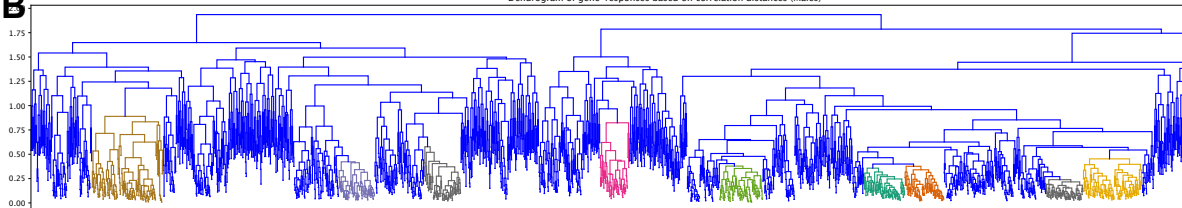
- A) For females
- B) For males

**A**

Dendrogram of gene-responses based on correlation distances (Females)

**B**

Dendrogram of gene-responses based on correlation distances (Males)



## Supplementary Citations

- Bjørndal, B. *et al.* (2015) A Phospholipid-Protein Complex from Antarctic Krill Reduced Plasma Homocysteine Levels and Increased Plasma Trimethylamine-N-Oxide (TMAO) and Carnitine Levels in Male Wistar Rats. *Mar. Drugs*, **13**, 5706–5721.
- van de Rest, O. *et al.* (2016) Metabolic effects of a 13-weeks lifestyle intervention in older adults: The Growing Old Together Study. *Aging (Albany, NY)*, **8**, 111–26.