Online Supplemental Material

# Amino acids, lipid metabolites and ferritin, as potential mediators linking red meat consumption to type 2 diabetes

**Contents**

**Tables**

**Supplementary Table 1:** Association of total red meat consumption with amino acid serum concentrations 2

**Supplementary Table 2:** Association of total red meat consumption with acylcarnitine serum concentrations 4

**Supplementary Table 3:** Association of total red meat consumption with diacyl-phosphatidylcholine serum concentrations 6

**Supplementary Table 4:** Association of total red meat consumption with acyl-alkyl-phosphatidylcholine serum concentrations 8

**Supplementary Table 5:** Association of total red meat consumption with lyso-phosphatidylcholine serum concentrations 10

**Supplementary Table 6:** Association of total red meat consumption with sphingomyelin serum concentrations 12

**Supplementary Table 7:** Association of preselected metabolites with the risk of developing type 2 diabetes 14

**Supplementary Table 8:** Association of total red meat consumption with serum concentrations of 126 metabolites and ferritin 16

**Figures**

**Supplementary Figure 1:** Workflow Mediation Analysis and Causal Diagram 1

**Supplementary Figure 2:** *p*-value-plot for amino acids 3

**Supplementary Figure 3:** *p*-value-plot for acylcarnitines 5

**Supplementary Figure 4:** *p*-value-plot for diacyl-phosphatidylcholines 7

**Supplementary Figure 5:** *p*-value-plot for acyl-alkyl-phosphatidylcholines 9

**Supplementary Figure 6:** *p*-value-plot for lysophosphatidylcholines 11

**Supplementary Figure 7:** *p*-value-plot for sphingomyelins 13

**Supplementary Figure 8:** Proportion explainable by biomarkers for subtypes of exposure (unprocessed and processed red meat) 15

****

**Figure 1 A** (Mediation Analysis): From the initial set of biomarkers potential mediators were selected based on four prespecified mediation criteria; mediation criteria were evaluated in regression models. **B** (Analytical design):The specific nutrient matrix provided by total red meat consumption (exposure) was assumed to influence endogenous metabolic processes as reflected in metabolic profiles which in turn were assumed to affect the risk of type 2 diabetes; biomarkers (lipids, amino acids, and ferritin) were included based on a plausible influence of red meat intake on circulating levels

**Supplementary Table 1:** Association of total red meat consumption with amino acid serum concentrations

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Amino Acids | F (n=1257) |  |  | M (n=790) |  |  |
| *β1* | *p*raw2 | *p*FDR3 | *β* | *p*raw | *p*FDR |
| Arginine | 0.022 | 0.489 | 0.683 | -0.033 | 0.394 | 0.668 |
| Glutamine | -0.035 | 0.263 | 0.460 | -0.059 | 0.130 | 0.364 |
| Glycine | -0.098 | 0.001 | 0.019 | -0.099 | 0.009 | 0.067 |
| Histidine | 0.048 | 0.118 | 0.330 | -0.046 | 0.233 | 0.465 |
| Methionine | 0.018 | 0.555 | 0.707 | -0.094 | 0.014 | 0.067 |
| Ornithine | 0.006 | 0.851 | 0.851 | -0.012 | 0.769 | 0.851 |
| Phenylalanine | 0.035 | 0.256 | 0.460 | -0.017 | 0.660 | 0.840 |
| Proline | -0.058 | 0.063 | 0.247 | -0.094 | 0.014 | 0.067 |
| Serine | 0.028 | 0.353 | 0.548 | 0.031 | 0.429 | 0.668 |
| Threonine | 0.056 | 0.071 | 0.247 | -0.055 | 0.157 | 0.365 |
| Tryptophan | -0.010 | 0.749 | 0.807 | 0.007 | 0.855 | 0.855 |
| Tyrosine | -0.015 | 0.611 | 0.713 | -0.067 | 0.075 | 0.262 |
| Valine | 0.041 | 0.179 | 0.417 | -0.018 | 0.637 | 0.840 |
| X-Leucine4 | 0.059 | 0.052 | 0.247 | -0.010 | 0.791 | 0.851 |

1Standardized beta coefficients (β) for a linear association of total red meat consumption with amino acid serum concentration in the subcohort (n=2,047). Regression models were adjusted for total energy intake [MJ/d], age [years], BMI [kg/m²], sports [h/week], biking [h/week], smoking (4 stages: never smoker, former smoker, current smoker <20 Units/day, current heavy smoker >20 Units/day), education (4 stages: no vocational training or in training, vocational training, technical school, technical college or university degree), antihypertensive medication (yes/no), antidyslipidemic medication (yes/no), intake of beverages (alcohol, coffee, sugar sweetened beverages) [g/day], and of whole grain bread, refined grain bread, butter, margarine, cabbage, cooked vegetables, mushrooms, potatoes, sauce, and poultry [g/MJ].

2Raw *p*-values and 3false discovery rate-controlled *p*-values (corrected within metabolite classes) from a two‑sided t‑test (H0: *β*=0). 4Sum of Leucine and Isoleucine.

**Supplementary Figure 2:** Manhattan-plot showing the negative decimal logarithm (‑LOG10) of false discovery rate-controlled *p*-values for the sex-stratified association between total red meat consumption and single amino acids concentrations; blue: men; magenta: women; solid line: significance (*p*<0.05) after Benjamini-Hochberg-correction; dashed line: trend (*p*<0.1) after Benjamini-Hochberg-correction.

**Supplementary Table 2:** Association of total red meat consumption with acylcarnitine serum concentrations

|  |  |  |
| --- | --- | --- |
| Acylcarnitines | F (n=1257) | M (n=790) |
| *β1* | *p*raw2 | *p*FDR3 | *β* | *p*raw | *p*FDR |
| C0 | 0.035 | 0.234 | 0.567 | 0.056 | 0.140 | 0.265 |
| C10:0 | 0.002 | 0.941 | 0.941 | 0.095 | 0.013 | 0.068 |
| C10:2 | 0.040 | 0.193 | 0.547 | 0.007 | 0.861 | 0.861 |
| C14:1 | -0.018 | 0.547 | 0.941 | 0.047 | 0.214 | 0.330 |
| C14:2 | 0.005 | 0.876 | 0.941 | 0.090 | 0.016 | 0.068 |
| C16:0 | -0.007 | 0.800 | 0.941 | 0.077 | 0.036 | 0.101 |
| C16:2 | 0.015 | 0.619 | 0.941 | 0.014 | 0.712 | 0.756 |
| C18:0 | 0.042 | 0.162 | 0.547 | 0.133 | <0.001 | 0.007 |
| C18:1 | 0.030 | 0.316 | 0.671 | 0.103 | 0.005 | 0.046 |
| C18:2 | 0.008 | 0.784 | 0.941 | 0.043 | 0.248 | 0.351 |
| C2:0 | 0.050 | 0.097 | 0.547 | 0.084 | 0.023 | 0.078 |
| C3:0 | 0.055 | 0.068 | 0.547 | 0.024 | 0.534 | 0.647 |
| (OH)C5\_(DC)C3(M) | 0.043 | 0.168 | 0.547 | 0.064 | 0.097 | 0.236 |
| (DC)C5\_(OH)C6 | 0.005 | 0.863 | 0.941 | 0.022 | 0.571 | 0.647 |
| (DC)C7 | 0.003 | 0.923 | 0.941 | 0.056 | 0.140 | 0.265 |
| C8:1 | 0.061 | 0.040 | 0.547 | 0.021 | 0.560 | 0.647 |
| C9:0 | -0.009 | 0.774 | 0.941 | -0.050 | 0.172 | 0.293 |

1Standardized beta coefficients (β) for a linear association of total red meat consumption with acylcarnitine serum concentrations in the subcohort (n=2,047). Regression models were adjusted for total energy intake [MJ/d], age [years], BMI [kg/m²], sports [h/week], biking [h/week], smoking (4 stages: never smoker, former smoker, current smoker <20 Units/day, current heavy smoker >20 Units/day), education (4 stages: no vocational training or in training, vocational training, technical school, technical college or university degree), antihypertensive medication (yes/no), antidyslipidemic medication (yes/no), intake of beverages (alcohol, coffee, sugar sweetened beverages) [g/day], and of whole grain bread, refined grain bread, butter, margarine, cabbage, cooked vegetables, mushrooms, potatoes, sauce, and poultry [g/MJ].

2Raw *p*-values and 3false discovery rate-controlled *p*-values (corrected within metabolite classes) from a two‑sided t‑test (H0: *β*=0).

**Supplementary Figure 3:** Manhattan-plot showing the negative decimal logarithm (‑LOG10) of false discovery rate-controlled *p*-values for the sex-stratified association between total red meat consumption and single amino acids concentrations; blue: men; magenta: women; solid line: significance (*p*<0.05) after Benjamini-Hochberg-correction; dashed line: trend (*p*<0.1) after Benjamini-Hochberg-correction.

**Supplementary Table 3:** Association of total red meat consumption with diacyl-phosphatidylcholine serum concentrations

|  |  |  |
| --- | --- | --- |
| Diacyl-phosphatidylcholines | F (n=1257) | M (n=790) |
| *β1* | *p*raw2 | *p*FDR3 | *β* | *p*raw | *p*FDR |
| C28:1 | -0.143 | <0.001 | <0.001 | -0.075 | 0.045 | 0.257 |
| C30:0 | -0.113 | <0.001 | 0.001 | -0.08 | 0.030 | 0.207 |
| C32:0 | -0.037 | 0.228 | 0.502 | 0.032 | 0.388 | 0.573 |
| C32:1 | -0.110 | <0.001 | 0.001 | -0.001 | 0.977 | 0.977 |
| C32:2 | -0.119 | <0.001 | 0.001 | -0.067 | 0.076 | 0.336 |
| C32:3 | -0.065 | 0.031 | 0.096 | -0.038 | 0.316 | 0.538 |
| C34:1 | -0.032 | 0.292 | 0.584 | 0.054 | 0.122 | 0.346 |
| C34:2 | -0.001 | 0.974 | 0.975 | 0.047 | 0.218 | 0.462 |
| C34:3 | -0.082 | 0.008 | 0.029 | 0.007 | 0.844 | 0.931 |
| C34:4 | -0.007 | 0.810 | 0.895 | 0.04 | 0.275 | 0.519 |
| C36:0 | 0.124 | <0.001 | 0.001 | 0.14 | <0.001 | 0.004 |
| C36:1 | -0.013 | 0.655 | 0.825 | 0.042 | 0.248 | 0.495 |
| C36:2 | 0.018 | 0.554 | 0.819 | 0.052 | 0.176 | 0.399 |
| C36:3 | -0.026 | 0.405 | 0.689 | 0.062 | 0.102 | 0.336 |
| C36:4 | 0.102 | 0.001 | 0.003 | 0.124 | 0.001 | 0.007 |
| C36:5 | -0.001 | 0.975 | 0.975 | 0.002 | 0.952 | 0.977 |
| C36:6 | -0.036 | 0.225 | 0.502 | -0.012 | 0.741 | 0.931 |
| C38:0 | 0.148 | <0.001 | <0.001 | 0.152 | <0.001 | 0.002 |
| C38:1 | 0.083 | 0.006 | 0.024 | 0.009 | 0.815 | 0.931 |
| C38:3 | -0.014 | 0.626 | 0.821 | 0.029 | 0.427 | 0.605 |
| C38:4 | 0.108 | <0.001 | 0.001 | 0.12 | 0.001 | 0.008 |
| C38:5 | -0.008 | 0.795 | 0.895 | 0.061 | 0.098 | 0.336 |
| C38:6 | 0.036 | 0.236 | 0.502 | 0.034 | 0.364 | 0.563 |
| C40:2 | 0.027 | 0.374 | 0.689 | 0.058 | 0.132 | 0.346 |
| C40:3 | 0.015 | 0.628 | 0.821 | 0.055 | 0.153 | 0.371 |
| C40:4 | 0.023 | 0.443 | 0.717 | 0.058 | 0.109 | 0.336 |
| C40:5 | -0.045 | 0.121 | 0.342 | 0.008 | 0.824 | 0.931 |
| C40:6 | 0.005 | 0.867 | 0.921 | -0.01 | 0.786 | 0.931 |
| C42:0 | -0.007 | 0.816 | 0.895 | -0.017 | 0.659 | 0.896 |
| C42:1 | 0.007 | 0.814 | 0.895 | 0.034 | 0.358 | 0.563 |
| C42:2 | -0.021 | 0.489 | 0.755 | -0.002 | 0.968 | 0.977 |
| C42:4 | 0.026 | 0.396 | 0.689 | 0.068 | 0.079 | 0.336 |
| C42:5 | 0.016 | 0.598 | 0.821 | 0.007 | 0.849 | 0.931 |
| C42:6 | -0.037 | 0.231 | 0.502 | -0.038 | 0.315 | 0.538 |

1Standardized beta coefficients (β) for a linear association of total red meat consumption with diacyl-phosphatidylcholine serum concentrations in the subcohort (n=2,047). Regression models were adjusted for total energy intake [MJ/d], age [years], BMI [kg/m²], sports [h/week], biking [h/week], smoking (4 stages: never smoker, former smoker, current smoker <20 Units/day, current heavy smoker >20 Units/day), education (4 stages: no vocational training or in training, vocational training, technical school, technical college or university degree), antihypertensive medication (yes/no), antidyslipidemic medication (yes/no), intake of beverages (alcohol, coffee, sugar sweetened beverages) [g/day], and of whole grain bread, refined grain bread, butter, margarine, cabbage, cooked vegetables, mushrooms, potatoes, sauce, and poultry [g/MJ].

2Raw *p*-values and 3false discovery rate-controlled *p*-values (corrected within metabolite classes) from a two‑sided t‑test (H0: *β*=0).



**Supplementary Figure 4:** Manhattan-plot of false discovery rate-corrected *p*-values for the sex-stratified association between total red meat consumption and single diacyl-phosphatidylcholines corrected for; blue: men; magenta: women.

**Supplementary Table 4:** Association of total red meat consumption with acyl-alkyl-phosphatidylcholine serum concentrations

|  |  |  |
| --- | --- | --- |
| Acyl‑alkylphosphatidylcholines | F (n=1257) | M (n=790) |
| *β1* | *p*raw2 | *p*FDR3 | *β* | *p*raw | *p*FDR |
| C30:0 | -0.120 | <0.001 | <0.001 | -0.066 | 0.063 | 0.136 |
| C30:1 | -0.051 | 0.098 | 0.165 | -0.021 | 0.588 | 0.679 |
| C30:2 | -0.059 | 0.046 | 0.098 | 0.014 | 0.712 | 0.798 |
| C32:1 | -0.005 | 0.878 | 0.920 | 0.056 | 0.135 | 0.250 |
| C32:2 | -0.031 | 0.290 | 0.370 | 0.028 | 0.447 | 0.570 |
| C34:0 | -0.097 | 0.001 | 0.002 | -0.073 | 0.037 | 0.091 |
| C34:1 | -0.091 | 0.002 | 0.005 | -0.039 | 0.282 | 0.417 |
| C34:2 | 0.095 | 0.001 | 0.005 | 0.078 | 0.036 | 0.091 |
| C34:3 | 0.066 | 0.023 | 0.064 | 0.106 | 0.004 | 0.014 |
| C36:0 | 0.031 | 0.311 | 0.372 | 0.125 | 0.001 | 0.003 |
| C36:1 | -0.099 | <0.001 | 0.002 | -0.079 | 0.026 | 0.080 |
| C36:2 | -0.047 | 0.094 | 0.165 | -0.036 | 0.308 | 0.438 |
| C36:3 | 0.127 | <0.001 | <0.001 | 0.137 | <0.001 | 0.002 |
| C36:4 | 0.235 | <0.001 | <0.001 | 0.205 | <0.001 | <0.001 |
| C36:5 | 0.183 | <0.001 | <0.001 | 0.225 | <0.001 | <0.001 |
| C38:0 | -0.024 | 0.435 | 0.502 | 0.046 | 0.228 | 0.367 |
| C38:1 | -0.039 | 0.200 | 0.274 | -0.051 | 0.185 | 0.325 |
| C38:2 | -0.042 | 0.154 | 0.236 | 0.027 | 0.474 | 0.572 |
| C38:3 | -0.045 | 0.131 | 0.211 | -0.010 | 0.790 | 0.836 |
| C38:4 | 0.129 | <0.001 | <0.001 | 0.146 | <0.001 | 0.001 |
| C38:5 | 0.211 | <0.001 | <0.001 | 0.219 | <0.001 | <0.001 |
| C38:6 | 0.155 | <0.001 | <0.001 | 0.156 | <0.001 | <0.001 |
| C40:1 | 0.042 | 0.159 | 0.236 | 0.121 | 0.001 | 0.005 |
| C40:2 | -0.060 | 0.040 | 0.097 | -0.007 | 0.851 | 0.875 |
| C40:3 | -0.082 | 0.006 | 0.019 | 0.010 | 0.791 | 0.836 |
| C40:4 | 0.058 | 0.048 | 0.098 | 0.119 | 0.002 | 0.006 |
| C40:5 | 0.051 | 0.088 | 0.163 | 0.042 | 0.261 | 0.402 |
| C40:6 | 0.029 | 0.312 | 0.372 | 0.033 | 0.375 | 0.514 |
| C42:1 | 0.041 | 0.185 | 0.263 | 0.136 | 0.000 | 0.002 |
| C42:2 | -0.061 | 0.042 | 0.097 | 0.029 | 0.442 | 0.570 |
| C42:3 | -0.037 | 0.213 | 0.281 | 0.078 | 0.040 | 0.091 |
| C42:4 | 0.007 | 0.798 | 0.869 | 0.046 | 0.212 | 0.357 |
| C42:5 | 0.062 | 0.035 | 0.093 | 0.060 | 0.110 | 0.214 |
| C44:3 | -0.014 | 0.647 | 0.725 | 0.082 | 0.034 | 0.091 |
| C44:4 | 0.000 | 0.993 | 0.993 | 0.026 | 0.479 | 0.572 |
| C44:5 | 0.053 | 0.075 | 0.145 | 0.061 | 0.102 | 0.210 |
| C44:6 | 0.004 | 0.895 | 0.920 | 0.001 | 0.987 | 0.987 |

1Standardized beta coefficients (β) for a linear association of total red meat consumption with acyl-alkyl-phosphatidylcholine serum concentrations in the subcohort (n=2,047). Regression models were adjusted for total energy intake [MJ/d], age [years], BMI [kg/m²], sports [h/week], biking [h/week], smoking (4 stages: never smoker, former smoker, current smoker <20 Units/day, current heavy smoker >20 Units/day), education (4 stages: no vocational training or in training, vocational training, technical school, technical college or university degree), antihypertensive medication (yes/no), antidyslipidemic medication (yes/no), intake of beverages (alcohol, coffee, sugar sweetened beverages) [g/day], and of whole grain bread, refined grain bread, butter, margarine, cabbage, cooked vegetables, mushrooms, potatoes, sauce, and poultry [g/MJ].

2Raw *p*-values and 3false discovery rate-controlled *p*-values (corrected within metabolite classes) from a two‑sided t‑test (H0: *β*=0).

 **Supplementary Figure 5:** Manhattan-plot of false discovery rate-corrected *p*-values for the sex-stratified association between total red meat consumption and single acyl-alkyl-phosphatidylcholines corrected for; blue: men; magenta: women.

**Supplementary Table 5:** Association of total red meat consumption with lyso-phosphatidylcholine serum concentrations

|  |  |  |
| --- | --- | --- |
| Lyso‑phosphatidylcholines | F (n=1257) | M (n=790) |
| *β1* | *p*raw2 | *p*FDR3 | *β* | *p*raw | *p*FDR |
| C14:0 | -0.083 | 0.007 | 0.017 | -0.070 | 0.064 | 0.213 |
| C16:0 | 0.009 | 0.776 | 0.780 | 0.045 | 0.233 | 0.466 |
| C16:1 | -0.110 | <0.001 | 0.002 | -0.002 | 0.955 | 0.955 |
| C17:0 | -0.057 | 0.043 | 0.087 | -0.098 | 0.004 | 0.019 |
| C18:0 | 0.014 | 0.637 | 0.780 | 0.034 | 0.372 | 0.620 |
| C18:1 | -0.008 | 0.780 | 0.780 | 0.044 | 0.224 | 0.466 |
| C18:2 | 0.011 | 0.706 | 0.780 | -0.007 | 0.849 | 0.946 |
| C20:3 | -0.012 | 0.696 | 0.780 | 0.023 | 0.538 | 0.768 |
| C20:4 | 0.109 | <0.001 | 0.002 | 0.115 | 0.002 | 0.017 |
| C28:1 | -0.105 | 0.001 | 0.002 | 0.007 | 0.851 | 0.946 |

1Standardized beta coefficients (β) for a linear association of total red meat consumption with lyso-phosphatidylcholine serum concentrations in the subcohort (n=2,047). Regression models were adjusted for total energy intake [MJ/d], age [years], BMI [kg/m²], sports [h/week], biking [h/week], smoking (4 stages: never smoker, former smoker, current smoker <20 Units/day, current heavy smoker >20 Units/day), education (4 stages: no vocational training or in training, vocational training, technical school, technical college or university degree), antihypertensive medication (yes/no), antidyslipidemic medication (yes/no), intake of beverages (alcohol, coffee, sugar sweetened beverages) [g/day], and of whole grain bread, refined grain bread, butter, margarine, cabbage, cooked vegetables, mushrooms, potatoes, sauce, and poultry [g/MJ].

2Raw *p*-values and 3false discovery rate-controlled *p*-values (corrected within metabolite classes) from a two‑sided t‑test (H0: *β*=0).



**Supplementary Figure 6:** Manhattan-plot of false discovery rate-corrected *p*-values for the sex-stratified association between total red meat consumption and single lyso-phosphatidylcholines corrected for; blue: men; magenta: women.

**Supplementary Table 6:** Association of total red meat consumption with sphingomyelin serum concentrations

|  |  |  |
| --- | --- | --- |
| Sphingomyelins | F (n=1257) | M (n=790) |
| *β1* | *p*raw2 | *p*FDR3 | *β* | *p*raw | *p*FDR |
| Hydroxy-C14:1 | -0.121 | <0.001 | <0.001 | -0.069 | 0.053 | 0.092 |
| Hydroxy-C16:1 | -0.071 | 0.010 | 0.040 | 0.000 | 0.998 | 0.998 |
| Hydroxy-C22:1 | -0.038 | 0.207 | 0.415 | 0.035 | 0.359 | 0.499 |
| Hydroxy-C22:2 | -0.040 | 0.171 | 0.399 | 0.013 | 0.725 | 0.846 |
| Hydroxy-C24:1 | -0.073 | 0.014 | 0.040 | 0.033 | 0.392 | 0.499 |
| C16:0 | -0.004 | 0.887 | 0.887 | 0.125 | 0.001 | 0.007 |
| C16:1 | -0.009 | 0.756 | 0.865 | 0.091 | 0.018 | 0.035 |
| C18:0 | 0.019 | 0.512 | 0.775 | 0.098 | 0.010 | 0.034 |
| C18:1 | 0.032 | 0.258 | 0.451 | 0.092 | 0.016 | 0.035 |
| C20:2 | -0.007 | 0.803 | 0.865 | -0.053 | 0.161 | 0.250 |
| C24:0 | -0.016 | 0.609 | 0.775 | 0.109 | 0.004 | 0.020 |
| C24:1 | 0.075 | 0.014 | 0.040 | 0.203 | <0.001 | <0.001 |
| C26:0 | -0.078 | 0.010 | 0.040 | -0.009 | 0.809 | 0.871 |
| C26:1 | 0.018 | 0.557 | 0.775 | 0.094 | 0.013 | 0.035 |

1Standardized beta coefficients (β) for a linear association of total red meat consumption with sphingomyelin serum concentrations in the subcohort (n=2,047). Regression models were adjusted for total energy intake [MJ/d], age [years], BMI [kg/m²], sports [h/week], biking [h/week], smoking (4 stages: never smoker, former smoker, current smoker <20 Units/day, current heavy smoker >20 Units/day), education (4 stages: no vocational training or in training, vocational training, technical school, technical college or university degree), antihypertensive medication (yes/no), antidyslipidemic medication (yes/no), intake of beverages (alcohol, coffee, sugar sweetened beverages) [g/day], and of whole grain bread, refined grain bread, butter, margarine, cabbage, cooked vegetables, mushrooms, potatoes, sauce, and poultry [g/MJ].

2Raw *p*-values and 3false discovery rate-controlled *p*-values (corrected within metabolite classes) from a two‑sided t‑test (H0: *β*=0).



**Supplementary Figure 7:** Manhattan-plot of false discovery rate-corrected *p*-values for the sex-stratified association between total red meat consumption and single sphingomyelins corrected for; blue: men; magenta: women.

**Supplementary Table 7:** Association of preselected metabolites with the risk of developing type 2 diabetes

|  |  |  |  |
| --- | --- | --- | --- |
| Selected Metabolites1 | HR (95%CI)2 | *p*raw3 | *p*FDR4 |
| Glycine5 | 0.66 (0.57, 0.77) | <0.001 | <0.001 |
| Diacyl PC C36:0 | 0.91 (0.82, 1.01) | 0.067 | 0.083 |
| Diacyl PC C36:4 | 1.20 (1.07, 1.35) | 0.002 | 0.003 |
| Diacyl PC C38:0 | 0.88 (0.79, 0.99) | 0.026 | 0.036 |
| Diacyl PC C38:4 | 1.24 (1.12, 1.38) | <0.001 | <0.001 |
| Acyl-Alkyl PC C34:0 | 1.03 (0.91, 1.17) | 0.600 | 0.600 |
| Acyl-Alkyl PC C34:2 | 0.73 (0.64, 0.83) | <0.001 | <0.001 |
| Acyl-Alkyl PC C34:3 | 0.55 (0.48, 0.64) | <0.001 | <0.001 |
| Acyl-Alkyl PC C36:1 | 0.96 (0.84, 1.09) | 0.527 | 0.554 |
| Acyl-Alkyl PC C36:3 | 0.78 (0.69, 0.88) | <0.001 | 0.000 |
| Acyl-Alkyl PC C36:4 | 0.96 (0.86, 1.07) | 0.483 | 0.534 |
| Acyl-Alkyl PC C36:5 | 0.84 (0.75, 0.94) | 0.001 | 0.003 |
| Acyl-Alkyl PC C38:4 | 0.88 (0.78, 0.99) | 0.036 | 0.047 |
| Acyl-Alkyl PC C38:5 | 0.84 (0.75, 0.94) | 0.003 | 0.005 |
| Acyl-Alkyl PC C38:6 | 0.87 (0.79, 0.97) | 0.014 | 0.021 |
| Acyl-Alkyl PC C40:4 | 0.80 (0.71, 0.91) | 0.001 | 0.002 |
| Lyso PC C17:0 | 0.78 (0.68, 0.89) | <0.001 | 0.000 |
| Lyso PC C20:4 | 0.95 (0.85, 1.07) | 0.428 | 0.499 |
| Hydroxy-SM C14:1 | 0.83 (0.73, 0.94) | 0.004 | 0.007 |
| SM C24:1 | 0.82 (0.74, 0.92) | 0.001 | 0.002 |
| Ferritin | 1.28 (1.15, 1.42) | <0.001 | <0.001 |

1Metabolites were selected based on the mediation criterion 2, i.e. a significant association with total red meat consumption in either, men or women and at least borderline‑significant associated in the other.

2Diabetes‑HR per SD in serum concentration; association of 21 preselected metabolites with type 2 diabetes risk was evaluated in Cox models in the case-cohort (n=2,681) adjusted for total red meat intake, total energy intake, age, BMI, sports, biking, smoking, education, antihypertensive medication, antidyslipidemic medication, intake of beverages (alcohol, coffee, sugar sweetened beverages), and consumption of whole grain bread, butter, margarine, light bread, cabbage, cooked vegetables, potatoes, and sauce.

3Raw *p*‑values and 4false discovery rate‑controlled *p*‑values (corrected for the 21 tests conducted among all metabolites that fulfilled mediation criterion 2) from a two sided Wald‑test.



 **Supplementary Figure 8:** Proportion explainable biomarkers of excess-risk associated with subtypes of red meat consumption. **A**: Unprocessed red meat consumption. **B**: Processed red consumption. Models were adjusted for age, sex, diet, lifestyle, and BMI. *Proportion of excess-risk explainable by biomarkers* was estimated as the difference of non-mediator-adjusted and mediator-adjusted HR divided by red meat-related excess diabetes risk; displayed are the median percentage, the IQR (grey box).Selected lipid-mediators: diacyl PC C38:4, lyso-PC C17:0, and hydroxy-SM C14:1; Selected mediators: lipid-mediators, ferritin, and glycine.

**Supplementary Table 8:** Association of total red meat consumption with serum concentrations of 126 metabolites and ferritin

|  |  |
| --- | --- |
|  | Pooled analysis (n=2,047) |
| Metabolite | *β1* | *p*raw2 | *p*FDR3 | *P*Bonferroni4 |
| Alkyl-Acyl PC C36:4 | 0.231 | 0.0001 | <0.001 | <0.001 |
| Alkyl-Acyl PC C38:5 | 0.219 | 0.0001 | <0.001 | <0.001 |
| Alkyl-Acyl PC C36:5 | 0.206 | 0.0001 | <0.001 | <0.001 |
| Alkyl-Acyl PC C38:6 | 0.164 | 0.0001 | <0.001 | <0.001 |
| Diacyl-PC C38:0 | 0.149 | 0.0001 | <0.001 | <0.001 |
| Diacyl-PC C36:0 | 0.135 | 0.0001 | <0.001 | <0.001 |
| Alkyl-Acyl PC C38:4 | 0.133 | 0.0001 | <0.001 | <0.001 |
| Alkyl-Acyl PC C36:3 | 0.128 | 0.0001 | <0.001 | <0.001 |
| SM C24:1 | 0.122 | 0.0001 | <0.001 | <0.001 |
| **Diacyl-PC C38:4** | 0.118 | 0.0001 | <0.001 | <0.001 |
| **Ferritin** | 0.116 | 0.0001 | <0.001 | <0.001 |
| LysoPC C20:4 | 0.115 | 0.0001 | <0.001 | <0.001 |
| **Diacyl-PC C36:4** | 0.111 | 0.0001 | <0.001 | <0.001 |
| **Hydroxy-SM C14:1** | -0.096 | 0.0001 | <0.001 | <0.001 |
| Alkyl-Acyl PC C30:0 | -0.099 | 0.0001 | <0.001 | <0.001 |
| Diacyl-PC C30:0 | -0.108 | 0.0001 | <0.001 | <0.001 |
| Diacyl-PC C28:1 | -0.112 | 0.0001 | <0.001 | <0.001 |
| Diacyl-PC C32:2 | -0.103 | 0.0001 | <0.001 | 0.002 |
| Alkyl-Acyl PC C34:0 | -0.089 | 0.0001 | <0.001 | 0.0042 |
| Alkyl-Acyl PC C36:1 | -0.085 | 0.0001 | <0.001 | 0.0068 |
| Alkyl-Acyl PC C34:2 | 0.088 | 0.0001 | <0.001 | 0.0089 |
| **Glycine** | -0.088 | 0.0002 | 0.001 | 0.0197 |
| Alkyl-Acyl PC C34:3 | 0.078 | 0.0005 | 0.003 | 0.0545 |
| AC C18:0 | 0.074 | 0.0009 | 0.005 | 0.0957 |
| LysoPC C14:0 | -0.079 | 0.0009 | 0.005 | 0.0902 |
| Alkyl-Acyl PC C40:1 | 0.078 | 0.001 | 0.005 | 0.1064 |
| Alkyl-Acyl PC C40:4 | 0.073 | 0.0011 | 0.005 | 0.1155 |
| Alkyl-Acyl PC C42:1 | 0.077 | 0.0013 | 0.006 | 0.1322 |
| Alkyl-Acyl PC C34:1 | -0.070 | 0.0013 | 0.006 | 0.1335 |
| **LysoPC C17:0** | -0.068 | 0.002 | 0.009 | 0.1978 |
| Alkyl-Acyl PC C36:0 | 0.067 | 0.0042 | 0.017 | 0.4109 |
| Diacyl-PC C32:1 | -0.063 | 0.006 | 0.024 | 0.5764 |
| AC C18:1 | 0.061 | 0.0063 | 0.024 | 0.5948 |
| AC C2:0 | 0.064 | 0.0067 | 0.025 | 0.6277 |
| LysoPC C28:1 | -0.062 | 0.0077 | 0.028 | 0.7187 |
| Proline | -0.058 | 0.0143 | 0.051 | 1 |
| LysoPC C16:1 | -0.057 | 0.0149 | 0.051 | 1 |
| Alkyl-Acyl PC C42:5 | 0.054 | 0.0169 | 0.057 | 1 |
| SM C18:1 | 0.052 | 0.0194 | 0.063 | 1 |
| Alkyl-Acyl PC C44:5 | 0.054 | 0.0199 | 0.063 | 1 |
| Alkyl-Acyl PC C40:3 | -0.051 | 0.021 | 0.064 | 1 |
| Diacyl-PC C38:1 | 0.055 | 0.0211 | 0.064 | 1 |
| Carnitine | 0.049 | 0.0231 | 0.067 | 1 |
| Diacyl-PC C32:3 | -0.052 | 0.0232 | 0.067 | 1 |
| AC C3:0 | 0.049 | 0.0275 | 0.078 | 1 |
| SM C18:0 | 0.049 | 0.0352 | 0.096 | 1 |
| Diacyl-PC C34:3 | -0.050 | 0.0361 | 0.096 | 1 |
| Alkyl-Acyl PC C40:5 | 0.049 | 0.0368 | 0.096 | 1 |
| SM C26:0 | -0.050 | 0.0369 | 0.096 | 1 |
| Hydroxy-SM C16:1 | -0.043 | 0.0461 | 0.117 | 1 |
| Alkyl-Acyl PC C30:1 | -0.047 | 0.0489 | 0.122 | 1 |
| SM C26:1 | 0.046 | 0.0512 | 0.125 | 1 |
| Alkyl-Acyl PC C36:2 | -0.041 | 0.0525 | 0.126 | 1 |
| Alkyl-Acyl PC C38:1 | -0.045 | 0.0558 | 0.131 | 1 |
| AC C8:1 | 0.043 | 0.0599 | 0.138 | 1 |
| SM C16:0 | 0.044 | 0.064 | 0.145 | 1 |
| Alkyl-Acyl PC C40:2 | -0.042 | 0.0654 | 0.145 | 1 |
| AC (OH)C5\_(DC)C3(M) | 0.044 | 0.0663 | 0.145 | 1 |
| AC C14:2 | 0.039 | 0.095 | 0.204 | 1 |
| Diacyl-PC C40:4 | 0.039 | 0.0962 | 0.204 | 1 |
| AC C10:0 | 0.039 | 0.0993 | 0.207 | 1 |
| Diacyl-PC C42:4 | 0.039 | 0.1022 | 0.209 | 1 |
| Diacyl-PC C42:6 | -0.038 | 0.1094 | 0.221 | 1 |
| X-Leucine4 | 0.036 | 0.1152 | 0.229 | 1 |
| Diacyl-PC C40:2 | 0.037 | 0.1193 | 0.233 | 1 |
| Diacyl-PC C38:6 | 0.036 | 0.122 | 0.235 | 1 |
| Alkyl-Acyl PC C40:6 | 0.034 | 0.1281 | 0.243 | 1 |
| Diacyl-PC C40:3 | 0.035 | 0.1454 | 0.271 | 1 |
| Diacyl-PC C36:2 | 0.035 | 0.1518 | 0.279 | 1 |
| Hydroxy-SM C24:1 | -0.033 | 0.1568 | 0.283 | 1 |
| Glutamine | -0.034 | 0.1584 | 0.283 | 1 |
| Alkyl-Acyl PC C38:3 | -0.031 | 0.164 | 0.289 | 1 |
| SM C24:0 | 0.032 | 0.1752 | 0.305 | 1 |
| LysoPC C18:0 | 0.031 | 0.1909 | 0.328 | 1 |
| AC C10:2 | 0.031 | 0.1935 | 0.328 | 1 |
| Alkyl-Acyl PC C30:2 | -0.029 | 0.2053 | 0.343 | 1 |
| AC C16:0 | 0.027 | 0.2094 | 0.345 | 1 |
| LysoPC C16:0 | 0.028 | 0.2301 | 0.375 | 1 |
| SM C20:2 | -0.027 | 0.242 | 0.389 | 1 |
| Tyrosine | -0.027 | 0.2483 | 0.394 | 1 |
| SM C16:1 | 0.025 | 0.2604 | 0.408 | 1 |
| Serine | 0.026 | 0.2643 | 0.409 | 1 |
| AC C9:0 | -0.025 | 0.2804 | 0.429 | 1 |
| AC (DC)C7 | 0.024 | 0.2997 | 0.453 | 1 |
| Valine | 0.023 | 0.3126 | 0.46 | 1 |
| Alkyl-Acyl PC C42:2 | -0.024 | 0.3147 | 0.46 | 1 |
| Diacyl-PC C36:6 | -0.024 | 0.3149 | 0.46 | 1 |
| AC C18:2 | 0.022 | 0.3214 | 0.464 | 1 |
| Diacyl-PC C38:5 | 0.022 | 0.3394 | 0.48 | 1 |
| Hydroxy-SM C22:2 | -0.021 | 0.3399 | 0.48 | 1 |
| Diacyl-PC C40:5 | -0.020 | 0.3823 | 0.531 | 1 |
| LysoPC C18:1 | 0.020 | 0.3847 | 0.531 | 1 |
| Threonine | 0.020 | 0.4081 | 0.554 | 1 |
| Diacyl-PC C34:2 | 0.020 | 0.4102 | 0.554 | 1 |
| Alkyl-Acyl PC C38:2 | -0.018 | 0.4304 | 0.575 | 1 |
| Alkyl-Acyl PC C44:3 | 0.018 | 0.4576 | 0.6 | 1 |
| Histidine | 0.018 | 0.4599 | 0.6 | 1 |
| Methionine | -0.018 | 0.4632 | 0.6 | 1 |
| AC C16:2 | 0.015 | 0.5261 | 0.675 | 1 |
| Diacyl-PC C42:1 | 0.014 | 0.5381 | 0.676 | 1 |
| Diacyl-PC C32:0 | -0.014 | 0.5414 | 0.676 | 1 |
| Alkyl-Acyl PC C42:4 | 0.013 | 0.547 | 0.676 | 1 |
| Diacyl-PC C42:0 | -0.014 | 0.5488 | 0.676 | 1 |
| Diacyl-PC C42:2 | -0.014 | 0.5536 | 0.676 | 1 |
| Alkyl-Acyl PC C32:1 | 0.013 | 0.5652 | 0.684 | 1 |
| Hydroxy-SM C22:1 | -0.013 | 0.5734 | 0.687 | 1 |
| Ornithine | 0.012 | 0.6035 | 0.716 | 1 |
| Diacyl-PC C42:5 | 0.012 | 0.6142 | 0.722 | 1 |
| Phenylalanine | 0.011 | 0.6399 | 0.746 | 1 |
| Diacyl-PC C36:1 | 0.010 | 0.6791 | 0.784 | 1 |
| LysoPC C20:3 | 0.008 | 0.72 | 0.798 | 1 |
| AC (DC)C5\_(OH)C6 | 0.009 | 0.7206 | 0.798 | 1 |
| Alkyl-Acyl PC C32:2 | -0.008 | 0.7267 | 0.798 | 1 |
| Alkyl-Acyl PC C38:0 | 0.008 | 0.7283 | 0.798 | 1 |
| Diacyl-PC C34:4 | 0.008 | 0.7312 | 0.798 | 1 |
| Diacyl-PC C36:3 | 0.008 | 0.7319 | 0.798 | 1 |
| Arginine | 0.008 | 0.7356 | 0.798 | 1 |
| AC C14:1 | 0.008 | 0.7479 | 0.801 | 1 |
| Diacyl-PC C38:3 | 0.007 | 0.7508 | 0.801 | 1 |
| LysoPC C18:2 | 0.007 | 0.7735 | 0.819 | 1 |
| Alkyl-Acyl PC C42:3 | 0.006 | 0.7928 | 0.832 | 1 |
| Diacyl-PC C34:1 | 0.005 | 0.8341 | 0.868 | 1 |
| Diacyl-PC C36:5 | 0.003 | 0.884 | 0.913 | 1 |
| Diacyl-PC C40:6 | 0.003 | 0.897 | 0.919 | 1 |
| Alkyl-Acyl PC C44:4 | 0.002 | 0.915 | 0.93 | 1 |
| Tryptophan | 0.002 | 0.9286 | 0.936 | 1 |
| Alkyl-Acyl PC C44:6 | 0.000 | 0.9956 | 0.996 | 1 |

1Standardized beta coefficients (β) for a linear association of total red meat consumption with serum concentrations of metabolites and ferritin in the subcohort (n=2,047). Regression models were adjusted for total energy intake [MJ/d], age [years], BMI [kg/m²], sports [h/week], biking [h/week], smoking (4 stages: never smoker, former smoker, current smoker <20 Units/day, current heavy smoker >20 Units/day), education (4 stages: no vocational training or in training, vocational training, technical school, technical college or university degree), antihypertensive medication (yes/no), antidyslipidemic medication (yes/no), intake of beverages (alcohol, coffee, sugar sweetened beverages) [g/day], and of whole grain bread, refined grain bread, butter, margarine, cabbage, cooked vegetables, mushrooms, potatoes, sauce, and poultry [g/MJ].

2Raw *p*-values; 3false discovery rate-controlled *p*-values; 3Bonferroni-corrected *p*-values; *p*-values from a two‑sided t‑test (H0: *β*=0) corrected over all metabolites and ferritin (127 tests). **Bold**: metabolites that were selected as mediators in the main analysis. Metabolites were ordered according to raw *p*-values.