**Supplementary material**

**Table S1:** Target and actual macronutrient composition for each intervention phase.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Macronutrient | Low fat diet | | High fat diet | | High protein diet | |
| Target | Actual | Target | Actual | Target | Actual |
| Fat %E | 30 | 29 | 45 | 44 | 30 | 29 |
| Carbohydrate %E | 55 | 55 | 40 | 42 | 40 | 40 |
| Protein %E | 15 | 16 | 15 | 14 | 30 | 31 |

%E, %Energy

**Table S2:** Characteristics of all participants (n=92) in response to the high fat diet.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Screening | LFD6 | HFD1 | HFD6 |
| Weight [kg] | 67.6 ± 11.8 | 66.6 ± 11.7§§§ | 66.5 ± 11.6§§§ | 67.0 ± 11.8§§†† |
| Glucose [mmol/L] | 4.78 ± 0.48 | 5.22 ± 0.81§§§ | 5.15 ± 0.62§§§ | 5.22 ± 0.61§§§ |
| Insulin [mU/L] | 5.21 ± 3.68 | 4.84 ± 3.17 | 5.70 ± 3.63 | 5.11 ± 3.52 |
| HOMA-IR | 1.07 ± 0.76 | 1.33 ± 1.08 | 1.42 ± 1.26§§§\* | 1.45 ± 1.13§§ |
| Glucagon [pmol/L] | 3.97 ± 2.71 | 2.06 ± 1.26§§§ | 3.04 ± 2.53§§§\* | 3.28 ± 2.37\*\*\*† |
| FFA [mmol/L] | 0.52 ± 0.26 | 0.61 ± 0.21§§§ | 0.58 ± 0.21§ | 0.50 ± 0.18\*\* |
| Total cholesterol [mmol/L] | 4.58 ± 0.93 | 4.29 ± 0.85§§§ | 4.47 ± 0.87\*\* | 4.70 ± 0.91\*\*\*†† |
| LDL [mmol/L] | 2.73 ± 0.77 | 2.59 ± 0.71§§ | 2.71 ± 0.73\*\* | 2.86 ± 0.79\*\*\* |
| HDL [mmol/L] | 1.38 ± 0.35 | 1.27 ± 0.33§§§ | 1.32 ± 0.34\*\*\* | 1.41 ± 0.37\*\*\*† |
| Triglycerides [mmol/L] | 0.99 ± 0.44 | 0.95 ± 0.43 | 0.89 ± 0.35 | 0.91 ± 0.37 |
| IHL [%] | n.a. | 2.1 ± 3.9 | 2.1 ± 3.9 | 2.3 ± 3.8 |

Values are shown as fasting mean ± SD. FFA, Free fatty acids; HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; HOMA-IR, homeostasis model assessment - estimated insulin resistance; IHL, intrahepatic lipid content; LFD6, investigation day after low fat diet for 6 weeks; n.a., not available

§p < 0.05 vs. Screening; §§p < 0.01 vs. Screening; §§§p < 0.001 vs. Screening; \*p < 0.05 vs. LFD6; \*\*p < 0.01 vs. LFD6; \*\*\* p < 0.001 vs. LFD6; †p < 0.05 vs. HFD1; ††p < 0.01 vs. HFD1

**Table S3:** Correlation analysis of clinical parameters with fasting glucagon.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Glucagon | | | | |
|  | Screening | LFD6 | HFD1 | HFD6 | HPD6 |
| Body weight | 0.109 | 0.174 | 0.102 | 0.109 | 0.221 |
| HbA1c | 0.107 | 0.027 | 0.194 | 0.000 | -0.127 |
| Glucose | -0.121 | 0.010 | **-0.254\*** | 0.071 | 0.016 |
| Insulin | **0.249\*** | 0.175 | 0.059 | 0.177 | 0.258 |
| HOMA-IR | **0.217\*** | 0.172 | 0.000 | 0.181 | 0.275 |
| Free fatty acids | **-0.241\*** | -0.003 | 0.145 | -0.094 | 0.070 |
| Total cholesterol | **-0.214\*** | -0.101 | -0.028 | -0.026 | -0.040 |
| LDL | -0.057 | -0.007 | 0.040 | 0.085 | -0.118 |
| HDL | **-0.234\*** | -0.185 | -0.144 | **-0.242\*** | 0.005 |
| Triglycerides | -0.157 | -0.107 | **-0.265\*\*** | 0.047 | 0.089 |
| IHL | n.a. | 0.195 | 0.148 | 0.121 | n.a. |

Shown is the correlation coefficient (Spearman or Pearson according to data distribution). For clinical investigation days Screening, LFD6, HFD1, HFD6 correlations were calculated with the data of all 92 participants, for HPD6 with the data of the 24 participants that continued with the high protein diet.

HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; HOMA-IR, Homeostasis model assessment - estimated insulin resistance; HPD6, investigation day after high protein diet for 6 weeks; IHL, intrahepatic lipid content; LFD6, investigation day after low fat diet for 6 weeks; n.a., not available

\*p < 0.05; \*\*p < 0.01

**Table S4:** Correlation analysis of the change (delta %) of fasting glucagon with the change of clinical parameters.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Delta % Glucagon | | | | |
|  | Scr – LFD6 | LFD6 – HFD1 | HFD1 – HFD6 | LFD6 – HFD6 | HFD6 – HPD6 |
| Delta % Body weight | -0.014 | -0.096 | -0.049 | 0.168 | -0.090 |
| Delta % HbA1c | -0.063 | 0.036 | -0.159 | 0.016 | -0.254 |
| Delta % Glucose | 0.121 | -0.150 | -0.105 | 0.031 | 0.262 |
| Delta % Insulin | 0.124 | -0.074 | -0.074 | 0.038 | 0.315 |
| Delta % HOMA-IR | 0.147 | -0.093 | 0.055 | 0.047 | **0.456\*** |
| Delta % FFA | -0.057 | **0.246\*** | 0.092 | 0.080 | -0.200 |
| Delta % Total Cholesterol | -0.025 | -0.006 | **-0.229\*** | 0.011 | -0.013 |
| Delta % LDL | -0.016 | -0.004 | -0.195 | 0.053 | -0.245 |
| Delta % HDL | -0.038 | 0.065 | **0.381\*\*\*** | -0.001 | 0.125 |
| Delta % Triglycerides | -0.059 | -0.023 | 0.002 | -0.112 | -0.103 |
| Delta % IHL | n.a. | 0.063 | 0.024 | 0.008 | n.a. |

Shown is the correlation coefficient (Spearman or Pearson according to data distribution). For the change between clinical investigation days Scr – LFD6, LFD6 – HFD1, HFD1 – HFD6, LFD6 – HFD6 correlations were calculated with the data of all 92 participants, for HFD6 – HPD6 with the data of the 24 participants that continued with the high protein diet.

FFA, Free fatty acids; HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; HOMA-IR, homeostasis model assessment - estimated insulin resistance; HPD6, investigation day after high protein diet for 6 weeks; IHL, intrahepatic lipid content; LFD6, investigation day after low fat diet for 6 weeks; n.a., not available; Scr, screening

\*p < 0.05; \*\*\*p < 0.001

**Table S5:** Changes of amino acids of all participants in response to the high fat diet.

|  |  |  |  |
| --- | --- | --- | --- |
|  | LFD6 | HFD1 | HFD6 |
| Total measured AA [µM] | 2223.7 ± 320.9 | 2229.7 ± 325.1 | 2239.6 ± 273.2 |
| Ala [µM] | 253.1 ± 60.2 | 261.3 ± 57.9 | 254.6 ± 56.2 |
| Arg [µM] | 71.3 ± 16.7 | 68.8 ± 12.9 | 69.3 ± 15.8 |
| Asn [µM] | 42.9 ± 7.0 | 41.4 ± 6.8 | 42.6 ± 6.1 |
| Asp [µM] | 7.3 ± 3.0 | 7.1 ± 2.9 | 7.0 ± 2.8\* |
| Cit [µM] | 23.5 ± 6.7 | 24.8 ± 7.0 | 25.0 ± 7.1 |
| Cystine [µM] | 15.3 ± 8.9 | 16.6 ± 9.3 | 15.5 ± 8.1 |
| Gln [µM] | 463.4 ± 90.9 | 457.4 ± 88.3 | 461.8 ± 80.4 |
| Glu [µM] | 121.2 ± 38.7 | 121.4 ± 30.2 | 113.9 ± 29.3 |
| Gly [µM] | 190.0 ± 55.9 | 195.3 ± 58.8 | 190.1 ± 53.3 |
| His [µM] | 76.5 ± 13.3 | 75.7 ± 11.2 | 75.6 ± 11.8 |
| Leu [µM] | 97.7 ± 19.6 | 98.8 ± 17.9 | 101.1 ± 18.6 |
| Lys [µM] | 143.7 ± 40.8 | 144.0 ± 34.5 | 146.0 ± 36.0 |
| Met [µM] | 19.1 ± 3.8 | 19.3 ± 3.6 | 19.7 ± 3.6 |
| Orn [µM] | 38.2 ± 13.4 | 37.3 ± 11.1 | 37.8 ± 14.4 |
| Phe [µM] | 45.6 ± 7.3 | 45.4 ± 6.2 | 45.9 ± 7.3 |
| Pro [µM] | 140.4 ± 51.3 | 157.4 ± 58.2\*\*\* | 146.8 ± 51.2†† |
| Ser [µM] | 93.0 ± 21.0 | 90.5 ± 19.8 | 91.4 ± 19.0 |
| Thr [µM] | 114.8 ± 32.9 | 109.1 ± 33.4 | 117.4 ± 35.5 |
| Trp [µM] | 45.6 ± 7.5 | 47.3 ± 8.0 | 47.5 ± 8.4\* |
| Tyr [µM] | 41.6 ± 10.4 | 43.4 ± 10.1 | 44.4 ± 11.1 |
| Val [µM] | 179.1 ± 60.2 | 183.1 ± 27.7 | 188.2 ± 33.2\* |

Values are shown as fasting mean ± SD. AA, amino acids; HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks

\*p < 0.05 vs. LFD6; \*\*p < 0.01 vs. LFD6; \*\*\* p < 0.001 vs. LFD6; †p < 0.05 vs. HFD1; ††p < 0.01 vs. HFD1

**Table S6:** Correlation analysis of fasting circulating amino acids with fasting glucagon.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Glucagon | | | |
|  | LFD6 | HFD1 | HFD6 | HPD6 |
| Total measured AA | 0.092 | -0.080 | **0.266\*** | -0.171 | |
| Ala | -0.004 | -0.094 | **0.239\*** | -0.198 | |
| Arg | 0.137 | -0.115 | 0.154 | -0.109 | |
| Asn | -0.044 | **-0.263\*** | -0.119 | -0.136 |
| Asp | -0.054 | -0.134 | -0.028 | 0.162 |
| Cit | **0.209\*** | -0.149 | 0.171 | -0.212 |
| Cystine | **0.289\*\*** | 0.065 | **0.296\*\*** | -0.312 |
| Gln | -0.038 | -0.089 | -0.009 | -0.024 |
| Glu | 0.165 | 0.125 | **0.260\*** | 0.343 |
| Gly | 0.116 | 0.022 | 0.164 | -0.267 |
| His | -0.087 | -0.164 | 0.071 | 0.309 |
| Leu | 0.099 | 0.117 | **0.313\*\*** | 0.175 |
| Lys | 0.024 | **-0.226\*** | 0.099 | 0.142 |
| Met | 0.007 | **-0.324\*\*** | 0.127 | -0.134 |
| Orn | **0.346\*\*** | 0.009 | **0.256\*** | -0.292 |
| Phe | 0.124 | 0.037 | **0.363\*\*** | 0.256 |
| Pro | **0.210\*** | 0.082 | **0.237\*** | -0.106 |
| Ser | 0.068 | **-0.231\*** | -0.007 | **-0.496\*** |
| Thr | **-0.207\*** | **-0.227\*** | -0.044 | -0.402 |
| Trp | 0.024 | -0.035 | 0.091 | 0.246 |
| Tyr | **0.225\*** | -0.004 | **0.263\*** | -0.099 |
| Val | 0.091 | **0.228\*** | **0.218\*** | 0.146 |

Shown is the correlation coefficient (Spearman or Pearson according to data distribution). For clinical investigation days LFD6, HFD1, HFD6 correlations were calculated with the data of all 92 participants, for HPD6 with the data of the 24 participants that continued with the high protein diet.

AA, amino acids; HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; HPD6, investigation day after high protein diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks

\*p < 0.05; \*\*p < 0.01

**Table S7:** Correlation analysis of the change (delta) of fasting glucagon with the change of fasting circulating amino acids.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Delta Glucagon | | | |
|  | LFD6 – HFD1 | HFD1 – HFD6 | LFD6 – HFD6 | HFD6 – HPD6 |
| Delta total measured AA | -0.204 | -0.209 | 0.039 | **-0.455\*** |
| Delta Ala | **-0.219\*** | -0.059 | 0.045 | -0.106 |
| Delta Arg | -0.129 | **-0.215\*** | -0.070 | -0.144 |
| Delta Asn | -0.082 | **-0.250\*** | 0.005 | -0.365 |
| Delta Asp | **-0.255\*** | 0.030 | -0.034 | -0.366 |
| Delta Cit | **-0.225\*** | **-0.219\*** | -0.061 | -0.370 |
| Delta Cystine | 0.173 | 0.003 | 0.104 | **-0.625\*\*** |
| Delta Gln | -0.187 | -0.096 | 0.087 | **-0.625\*\*** |
| Delta Glu | 0.006 | 0.043 | -0.076 | 0.139 |
| Delta Gly | -0.065 | -0.078 | 0.013 | **-0.539\*\*** |
| Delta His | -0.128 | -0.032 | 0.099 | 0.129 |
| Delta Leu | -0.118 | -0.142 | **0.229\*** | 0.064 |
| Delta Lys | -0.062 | -0.128 | -0.062 | -0.214 |
| Delta Met | **-0.245\*** | -0.134 | **0.257\*** | -0.295 |
| Delta Orn | -0.049 | -0.175 | -0.019 | -0.328 |
| Delta Phe | -0.100 | -0.053 | **0.241\*** | -0.035 |
| Delta Pro | -0.066 | -0.080 | 0.093 | -0.288 |
| Delta Ser | **-0.290\*\*** | -0.208 | -0.078 | -0.255 |
| Delta Thr | -0.158 | -0.206 | -0.028 | -0.116 |
| Delta Trp | 0.024 | -0.112 | 0.118 | -0.202 |
| Delta Tyr | -0.097 | -0.124 | 0.061 | -0.189 |
| Delta Val | -0.110 | -0.103 | 0.078 | -0.221 |

Shown is the correlation coefficient (Spearman or Pearson according to data distribution). For the change between clinical investigation days LFD6 – HFD1, HFD1 – HFD6, LFD6 – HFD6 correlations were calculated with the data of all 92 participants, for HFD6 – HPD6 with the data of the 24 participants that continued with the high protein diet.

AA, amino acids; HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; HPD6, investigation day after high protein diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks.

\*p < 0.05; \*\*p < 0.01

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**Figure S1: Glucagon changes in age subgroups.** To analyze whether the age of the subjects had an impact on high fat diet induced GCGN changes the cohort was split in three age subgroups (tertiles: 1. 18-23 years, n=36 2. 24-30 years, n=26 3. 31-70 years, n=30). Values are shown as mean ± SEM. As all groups showed a GCGN decrease with the LFD and an increase with the HFD, the induced changes of GCGN seem to occur independent from age. HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks; Scr, Screening visit.

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**Figure S2: Glucagon changes grouped by sex.** The GCGN levels were significantly higher in males (n=34) compared to females (n=58) at baseline (grey), after 6 weeks of LFD (green) and after 6 weeks of HFD (orange). Values are shown as mean ± SEM. However, both sexes showed a significant GCGN decrease after 6 weeks of LFD and a significant increase of GCGN levels in response to 6 weeks of HFD. HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks; Scr, Screening visit.

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**Figure S3: Glucagon levels before and after a test meal.** GCGN was measured before (0 min) and after (240 min) the consumption of a test meal in a subgroup of 14 participants randomly taken from the entire study group. Repeated measures ANOVA followed by Bonferroni adjusted post hoc test was performed separately for each time point (0 min and 240 min). Values are shown as mean ± SEM. In this subgroup the GCGN levels before intake of the test meal increased slightly, but not significant in response to the high dietary fat intake. The postprandial GCGN increased progressively and was significantly higher after 6 weeks of HFD compared to LFD. HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks.

**Table S8:** Characteristics of the subgroup of participants that continued with the high protein intervention (n=24) at each clinical investigation day.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Screening | LFD6 | HFD1 | HFD6 | HPD6 |
| Weight [kg] | 72.6 ± 12.8 | 71.8 ± 12.1§ | 71.7 ± 12.2§ | 72.4 ± 12.5 | 72.5 ± 12.9 |
| Glucose [mmol/L] | 5.05 ± 0.46 | 5.61 ± 1.01 | 5.24 ± 0.71 | 5.49 ± 0.57 | 5.75 ± 0.67 |
| Insulin [mU/L] | 5.38 ± 4.00 | 5.25 ± 4.01 | 6.19 ± 5.05 | 5.88 ± 4.41 | 4.07 ± 2.92† |
| HOMA-IR | 1.07 ± 0.76 | 1.33 ± 1.08 | 1.42 ± 1.26 | 1.45 ± 1.13 §§ | 1.03 ± 0.72# |
| GCGN [pmol/L] | 3.21 ± 2.75 | 2.10 ± 1.12 | 2.99 ± 2.09 | 3.43 ± 2.48\* | 4.55 ± 1.49 §\*\*\* ††† |
| FAA [mmol/L] | 0.47 ± 0.22 | 0.58 ± 0.25 | 0.54 ± 0.19 | 0.46 ± 0.16 | 0.60 ± 0.14§ |
| TC [mmol/L] | 4.69 ± 0.98 | 4.38 ± 0.92§ | 4.41 ± 1.05 | 4.82 ± 1.05\*\* | 4.38 ± 0.81# |
| LDL [mmol/L] | 2.89 ± 0.83 | 2.74 ± 0.76 | 2.71 ± 0.86 | 2.97 ± 0.91 | 2.73 ± 0.66 |
| HDL [mmol/L] | 1.32 ± 0.34 | 1.18 ± 0.29 §§§ | 1.21 ± 0.36 | 1.36 ± 0.42 \*\*\*† | 1.22 ± 0.32§## |
| TG [mmol/L] | 0.94 ± 0.38 | 1.01 ± 0.45 | 0.87 ± 0.33 | 0.92 ± 0.42 | 0.94 ± 0.68 |

Values are shown as fasting mean ± SD. FFA, Free fatty acids; GCGN, Glucagon; HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; HOMA-IR, homeostasis model assessment - estimated insulin resistance; HPD6, investigation day after high protein diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks; TC, Total cholesterol; TG, Triglycerides

§p < 0.05 vs. Screening; §§p < 0.01 vs. Screening; §§§p < 0.001 vs. Screening; \*p < 0.05 vs. LFD6; \*\*p < 0.01 vs. LFD6; \*\*\* p < 0.001 vs. LFD6; †p < 0.05 vs. HFD1; ††p < 0.01 vs. HFD1; ††† p < 0.001 vs. HFD1; # p < 0.05 vs. HFD6; ## p < 0.01 vs. HFD6; ###p < 0.001 vs. HFD6

**Table S9:** Changes of fasting amino acids in the subgroup of participants that continued with the high protein intervention (n=24).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | LFD6 | HFD1 | HFD6 | HPD6 |
| Total measured AA [µM] | 2360.8 ± 205.3 | 2380.3 ± 239.7 | 2335.5 ± 258.6 | 2353.2 ± 239.1 |
| Ala [µM] | 256.2 ± 61.6 | 277.6 ± 56.6 | 260.8 ± 67.2 | 232.4 ± 54.4\*†††## |
| Arg [µM] | 78.1 ± 16.0 | 70.7 ± 14.3 | 72.7 ± 12.3 | 71.8 ± 12.5 |
| Asn [µM] | 42.6 ± 6.9 | 39.2 ± 6.6 | 39.9 ± 5.5 | 41.2 ± 6.1 |
| Asp [µM] | 8.6 ± 3.1 | 8.0 ± 3.3 | 7.8 ± 3.3 | 9.7 ± 3.1 |
| Cit [µM] | 24.1 ± 6.2 | 24.6 ± 6.7 | 24.2 ± 7.4 | 27.6 ± 9.1 |
| Cystine [µM] | 16.7 ± 7.4 | 18.6 ± 8.8 | 15.4 ± 7.0 | 18.0 ± 7.3 |
| Gln [µM] | 509.6 ± 70.7 | 509.6 ± 83.8 | 484.8 ± 77.8 | 449.7 ± 65.6\*\*†† |
| Glu [µM] | 123.8 ± 36.1 | 124.9 ± 32.4 | 112.9 ± 24.1† | 125.1 ± 26.7# |
| Gly [µM] | 205.1 ± 45.8 | 218.5 ± 59.1 | 205.1 ± 42.5 | 178.4 ± 40.2\*††# |
| His [µM] | 80.3 ± 10.2 | 75.2 ± 10.4 | 77.1 ± 12.7 | 79.0 ± 11.1 |
| Leu [µM] | 102.5 ± 14.5 | 101.4 ± 16.9 | 103.7 ± 18.2 | 114.5 ± 21.1\*† |
| Lys [µM] | 160.8 ± 30.3 | 161.6 ± 34.9 | 164.2 ± 32.7 | 182.8 ± 38.6\*† |
| Met [µM] | 20.9 ± 3.5 | 20.6 ± 3.7 | 20.2 ± 4.0 | 23.4 ± 4.0# |
| Orn [µM] | 42.4 ± 11.8 | 39.7 ± 9.7 | 43.5 ± 20.4 | 41.6 ± 11.9 |
| Phe [µM] | 46.4 ± 4.6 | 44.8 ± 6.0 | 44.7 ± 6.2 | 51.1 ± 4.5\*\*††## |
| Pro [µM] | 156.2 ± 61.4 | 177.0 ± 69.5\* | 167.8 ± 65.2 | 156.8 ± 65.7† |
| Ser [µM] | 94.3 ± 17.7 | 90.6 ± 21.1 | 92.9 ± 20.8 | 97.2 ± 14.6 |
| Thr [µM] | 108.2 ± 33.4 | 99.6 ± 33.9 | 102.5 ± 25.7 | 114.3 ± 26.9† |
| Trp [µM] | 47.3 ± 8.3 | 47.1 ± 7.8 | 47.9 ± 9.7 | 55.5 ± 8.6\*\*††# |
| Tyr [µM] | 46.5 ± 8.4 | 46.8 ± 8.9 | 50.3 ± 10.6 | 56.3 ± 10.3 |
| Val [µM] | 190.3 ± 30.0 | 184.1 ± 27.9 | 197.2 ± 36.4 | 226.6 ± 32.6\*\*†††## |

Values are shown as fasting mean ± SD. AA, amino acids; HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; HPD6, investigation day after high protein diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks; \*p < 0.05 vs. LFD6; \*\*p < 0.01 vs. LFD6; \*\*\* p < 0.001 vs. LFD6; †p < 0.05 vs. HFD1; ††p < 0.01 vs. HFD1; ††† p < 0.001 vs. HFD1; # p < 0.05 vs. HFD6; ## p < 0.01 vs. HFD6; ###p < 0.001 vs. HFD6

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A:0.000

C:0.353

E:0.647

Monozygotic (MZ)

Dizygotic (DZ)

A: 0,0

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A:0.338

C:0.293

E:0.369

A:0.452

C:0.082

E.0.466

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Dizygotic (DZ)

Monozygotic (MZ)

A:0.000

C:0.226  
E:0.774

**Figure S4: Heritability of Glucagon.** Heritability was estimated by applying the “ACE structural equation model” for each clinical investigation day (CID). The x and y axis show plasma GCGN in pmol/L. A, additive genetic influences; C, common environmental influences; E, individual environmental influences; HFD1, investigation day after high fat diet for 1 week; HFD6, investigation day after high fat diet for 6 weeks; LFD6, investigation day after low fat diet for 6 weeks.