



Supplementary figure 1 – the procedure of an experiment session.



Supplementary Figure 2 – participant recruitment flowchart. aGLP-1, active GLP-1.



Supplementary figure 3 – postprandial glucose and insulin results of all test drinks with milk and sugar added. A: postprandial glucose levels of plain water, espresso, instant, and boiled coffee. B: postprandial glucose levels of plain water, decaffeinated coffee, and water with milk and sugar. C: glucose iAUC of all test drinks. D: postprandial insulin levels of plain water, espresso, instant, and boiled coffee. E: postprandial insulin levels of plain water, decaffeinated coffee, and water with milk and sugar. F: insulin iAUC of all test drinks. For all figures, n = 21 and values are estimated marginal means adjusted for fasting levels and usual coffee consumption frequency. Error bars depict SEM. Statistical significance is set at p < 0.05 and results of all statistical tests are adjusted for multiple comparisons. For A, B, D, E, statistically significant differences in timepoint measurements between test drinks are indicated by the following symbols: \*, instant coffee and plain water. †, boiled coffee and plain water.  $\pm$ , espresso coffee and plain water. S, decaffeinated coffee and plain water. I, water with milk and sugar and plain water. For C and F, bars with different letters are significantly different. Decaf, decaffeinated coffee; Milk w/ sugar, water with milk and sugar. iAUC, incremental area-undercurve.



Supplementary figure 4 – postprandial glucose and insulin results of test drinks without milk and sugar added (i.e. black coffees). A: postprandial glucose levels of all test drinks. B: glucose iAUC of all test drinks. C: postprandial insulin levels of all test drinks. D: insulin iAUC of all test drinks. For all figures, n = 10 and values are estimated marginal means adjusted for fasting levels and usual coffee consumption frequency. Error bars depict SEM. Statistical significance is set at p < 0.05 and results of all statistical tests are adjusted for multiple comparisons. For A and C, statistically significant differences in timepoint measurements between test drinks are indicated by the following symbols: \*, instant coffee and plain water. +, boiled coffee and plain water. +, espresso coffee and plain water. §, decaffeinated coffee and plain water. No significant difference in glucose and insulin iAUC was found between different test drinks. Decaf, decaffeinated coffee. iAUC, incremental area-under-curve.



Supplementary figure 5 – postprandial glucose (A-D) and insulin (F-I) levels of black coffees and coffees with milk and sugar added, tested using espresso, instant, boiled, and decaffeinated coffee. Solid lines represent black coffee, while dashed lines represent coffee with milk and sugar added. For all figures, n = 10 and values are estimated marginal means adjusted for fasting measurements and usual coffee consumption frequency. Error bars depict SEM. Asterisks depict statistically significant differences in timepoint measurements between coffees with and without milk and sugar added, p < p0.05. E & J: glucose and insulin iAUC, respectively, after preloading black coffees and coffees with milk and sugar added, tested using the espresso, instant, boiled, and decaffeinated coffee. Grey bars represent values of coffee with milk and sugar, while black bars represent values of black coffee. Error bars depict SEM. Asterisks depict statistically significant difference between black coffees and coffees with milk and sugar added, p < 0.05. Results of all statistical tests are adjusted for multiple comparisons. No statistically significant differences in insulin iAUC were observed between any pair of coffees. Decaf, decaffeinated coffee. iAUC, incremental area-under-curve.



Supplementary figure 6 – postprandial active GLP-1 results of test drinks with milk and sugar added. A: postprandial active GLP-1 levels of plain water, espresso, instant, and boiled coffee. B: postprandial active GLP-1 levels of plain water, decaffeinated coffee, and water with milk and sugar added. C: iAUC of active GLP-1 of all test drinks. For all figures, n = 12 and values are estimated marginal means adjusted for fasting levels and usual coffee consumption frequency. Error bars depict SEM. After adjusting results for multiple comparisons, no significance differences were observed. GLP-1, glucagon-like peptide-1. Decaf, decaffeinated coffee. iAUC, incremental area-under-curve.



Supplementary figure 7 – postprandial active GLP-1 results of test drinks with and without milk and sugar added. A: postprandial active GLP-1 levels of all test drinks. B: postprandial active GLP-1 levels of plain water, decaffeinated coffee, and water with milk and sugar added. C: iAUC of active GLP-1 of all test drinks. For all figures, n = 8 and values are estimated marginal means adjusted for fasting levels and usual coffee consumption frequency. Error bars depict SEM. No significant differences were observed before adjusting for multiple comparisons. GLP-1, glucagon-like peptide-1. Decaf, decaffeinated coffee. iAUC, incremental area-under-curve.



Supplementary Figure 8 – postprandial nitrotyrosine results of test drinks with and without milk and sugar added. A: postprandial nitrotyrosine levels of plain water and all test drinks with milk and sugar added. B: postprandial nitrotyrosine levels of plain water and all coffees without milk and sugar added. C-F : postprandial nitrotyrosine levels of espresso, instant, boiled, and decaffeinated coffees, respectively, with and without milk and sugar added. For all figures, n = 21 and values are estimated marginal means adjusted for fasting levels and usual coffee consumption frequency. Error bars depict SEM. No significant differences were observed before adjusting for multiple comparisons. Decaf, decaffeinated coffee.



§∥

60

Time (min)

§∥

90

D

0

0



30

Ε

40

30

lnsulin (µU/ml) 05

10

0

0

120

§∥

30

-----Water ···•·· Espresso ----Instant ···•·· Boiled

90

60

Time (min)





Supplementary figure 9 – postprandial glucose and insulin results of all test drinks with milk and sugar added, with data from dropouts included. A: postprandial glucose levels of plain water, espresso, instant, and boiled coffee. B: postprandial glucose levels of plain water, decaffeinated coffee, and water with milk and sugar. C: glucose iAUC of all test drinks. D: postprandial insulin levels of plain water, espresso, instant, and boiled coffee. E: postprandial insulin levels of plain water, decaffeinated coffee, and water with milk and sugar. F: insulin iAUC of all test drinks. For all figures, values are estimated marginal means adjusted for fasting levels and usual coffee consumption frequency. Error bars depict SEM. Statistical significance is set at p < 0.05 and results of all statistical tests are not adjusted for multiple comparisons. For A, B, D, E, statistically significant differences in timepoint measurements between test drinks are indicated by the following symbols: \*, instant coffee and plain water. †, boiled coffee and plain water. ‡, espresso coffee and plain water. §, decaffeinated coffee and plain water. II, water with milk and sugar and plain water. For C and F, bars with different letters are significantly different. Decaf, decaffeinated coffee; Milk w/ sugar, water with milk and sugar. iAUC, incremental area-under-curve.



Time (min)

Time (min)

120

sugar

Supplementary figure 10 – postprandial glucose and insulin results of all test drinks with milk and sugar added, with data from dropouts included. A: postprandial glucose levels of plain water, espresso, instant, and boiled coffee. B: postprandial glucose levels of plain water, decaffeinated coffee, and water with milk and sugar. C: glucose iAUC of all test drinks. D: postprandial insulin levels of plain water, espresso, instant, and boiled coffee. E: postprandial insulin levels of plain water, decaffeinated coffee, and water with milk and sugar. F: insulin iAUC of all test drinks. For all figures, values are estimated marginal means adjusted for fasting levels and usual coffee consumption frequency. Error bars depict SEM. Statistical significance is set at p < 0.05 and results of all statistical tests are adjusted for multiple comparisons. For A, B, D, E, statistically significant differences in timepoint measurements between test drinks are indicated by the following symbols: \*, instant coffee and plain water. †, boiled coffee and plain water. ‡, espresso coffee and plain water. §, decaffeinated coffee and plain water. II, water with milk and sugar and plain water. For C and F, bars with different letters are significantly different. Decaf, decaffeinated coffee; Milk w/ sugar, water with milk and sugar. iAUC, incremental area-under-curve.