Supplements

Supplementary methods S1: Metabolic network model

$$\begin{aligned}\frac{dPyr}{dt} &= k_1 \cdot c_{Glc} + k_{2b} \cdot L + k_5 \cdot c_{Ala} - (k_{2f} + k_3 + k_{d,Pyr}) \cdot c_{Pyr} \\ \frac{dCit}{dt} &= k_3 \cdot c_{Pyr} - (k_4 + k_{d,Cit}) \cdot c_{Cit} \\ \frac{dLac}{dt} &= k_{2f} \cdot c_{Pyr} - (k_{2b} + k_{d,Lac}) \cdot c_{Lac} \\ \frac{dGlu}{dt} &= k_4 \cdot c_{Cit} - k_{d,Glu} \cdot c_{Glu}\end{aligned} \quad (3)$$

where c represent the metabolite concentrations (pyruvate M3 (excluded from the fitting process due to low quality of pyruvate measurement), citrate_M2, lactate_M3, glutamate_M2, glucose_M6 and alanine_M3), k represent the rates of the network and k_d represents the outgoing fluxes. Glucose M6 and alanine M3 were interpolated from data and treated as input to the model (Fig. 7).

The model was fitted to individual subject data in two scenarios. Firstly, assuming that the rate constants in the model were not changed by the interventions, the model was fitted to the control, GG2 and GG4 data simultaneously. The quality of the fit here serves as a baseline, referred to as Model 1 [1] in the following, to compare the results from the other scenario to. In the second scenario, one of the six rate constants was assumed to be influenced by the intervention and thus takes different values for the control, GG2 and GG4 in the fitting procedure (referred to as Model 2 [2] to Model 7 [7] in the following). Please note that the simulated quantities are in principle concentration in cells, which are assumed to be proportional to the blood compartment from which the samples were collected. Therefore four scaling factors, connecting cellular levels to blood levels, were included as free parameters in the fitting procedure. The fitting procedure was guided by minimizing the root mean square (RMS) difference between model simulation (y) and the measured data points ($\hat{y}$).

$$RMS = \sqrt{\sum_{i=1}^{n} \frac{(y_i - \hat{y}_i)^2}{\sigma_i^2}} \quad (4)$$

where σ denotes the standard deviation of the measured data point, and n denotes the number of data points.

A differential evolution based global optimizer was employed for fitting. We allow a large parameter space for the rate constants while the scaling factors are limited in their physiological range. Each optimization task was repeated 50 times, among which the best result was used to calculate the Akaike information criterion with correction (AICc)[1]◎ for model comparison (Eq. 5). The AICc evaluates model quality based on both quality of fitting and the number of free parameters. 2 units larger in AICc means 0.368 times as probable as the model having smaller AICc.

$$AICc = 2P + RMS^2 + N\log(2\pi) + 2\frac{P(P+1)}{N-P-1} \quad (5)$$

where P is the number of fitted parameters, N is the number of data points.

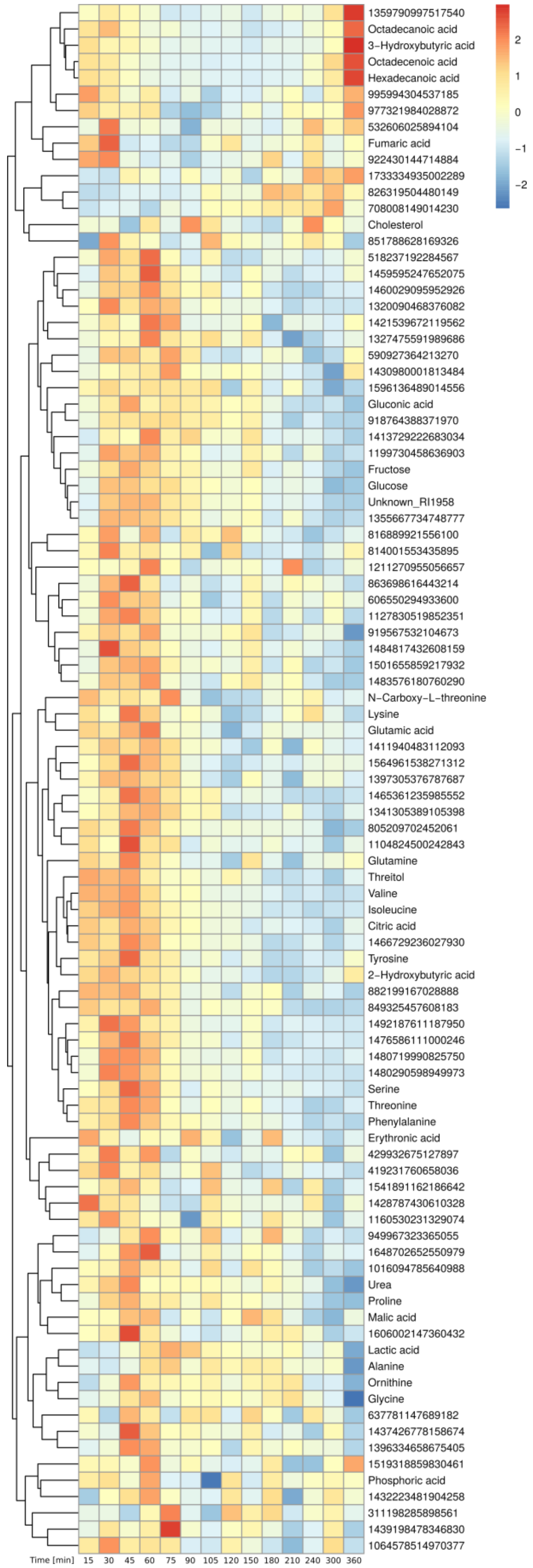
Supplementary Table S1: Results for glucose, insulin and incretin responses (Mean values and percentage differences; 95% confidence intervals vs. control)**[2]**⚜

		GG2			GG4		
	Control	Mean	% Difference	CI	Mean	% Difference	CI
Glucose AUC_{0-120}	148.9	128.4	-13.8	-27.3, 2.3	110.4	-25.9	-37.8, -11.7
Insulin AUC_{0-120}	2322.4	1941.6	-16.4	-27.9, 3.1	1787.7	-23.0	-33.8, -10.5
Insulin AUC_{0-2400}	3541.8	3008.6	-15.1	-24.1, -4.9	2749.7	-22.4	-30.8, -12.9
GIP AUC_{0-120}	2592.9	2437.8	-6.0	-16.3, 5.6	2323.9	-10.4	-20.2, 0.7
GIP AUC_{0-240}	4862.5	4693.3	-3.5	-13.4, 7.6	4568.2	-6.1	-15.7, 4.7
GLP-1 AUC_{0-120}	1579.2	1513.9	-4.1	-15.6, 8.9	1553.1	-1.7	-13.4, 11.7
GLP-1 AUC_{0-240}	3136.6	3057.6	-2.5	-12.0, 8.0	3062.8	-2.4	-11.9, 8.2

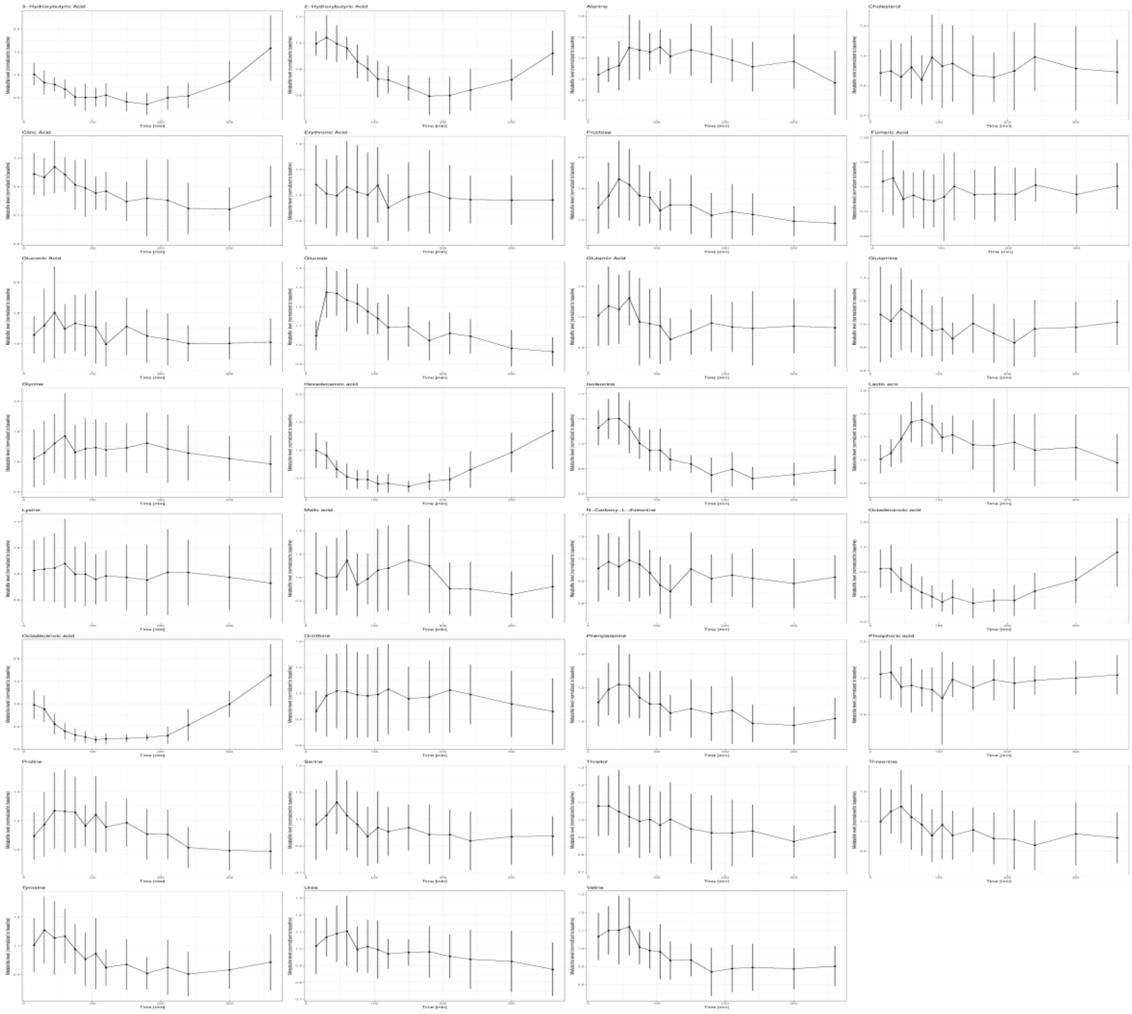
Supplementary Table S2: Single metabolite model – Computed appearance rates for the three conditions (Ctrl, GG2 and GG4) and all 12 subjects separately

Interventior	Subject	Glc_M6	Lac_M3	Ala_M3	Cit_M2	Glu_M2	Gly_M2	Ser_M3	Glu_M5	Gln_M5	Val_M5	Tyr_M9	Ile_M6	Lys_M6	Thr_M4
Ctrl	**S01**	0.268625	0.0113015	0.00261828	0.00071489	0.00066921	0.00283942	0.00441484	0.0045482	0.00518692	0.0040232	0.00633906	0.00367466	0.00453214	0.00376696
Ctrl	**S02**	0.0220079	0.0020506	0.00029516	0.00016284	0.00045391	0.0005323	0.00027139	0.00075082	0.00030813	0.00018462	0.00030654	0.00078227	0.00092534	0.00031617
Ctrl	**S03**	0.0492555	0.00464128	0.00155975	0.00045729	0.00147616	0.00182907	0.00312067	0.00342361	0.00436252	0.0021863	0.00417276	0.00328131	0.459228	0.00249909
Ctrl	**S04**	0.0421099	0.004998	0.0012249	0.00017307	0.00029756	0.00095313	0.00044609	0.00168713	0.00144763	0.0005681	0.00199683	0.00193874	0.00050656	0.00095608
Ctrl	**S05**	0.0795471	0.00403656	0.00113356	0.00069206	0.00019899	0.00183711	0.00454317	0.00308448	0.00343227	0.00255536	0.00408834	0.00258602	0.00658707	0.00341798
Ctrl	**S06**	0.0546088	0.00534517	0.00052673	0.00014183	0.00051134	0.00066988	0.00017879	0.00208816	0.00267133	0.00129069	0.00278842	0.00124845	0.00223881	0.00169152
Ctrl	**S07**	0.0452823	0.00457272	0.00112152	0.00052634	0.00055371	0.00149617	0.00102719	0.00328338	0.00424853	0.00194819	0.00315311	0.00179443	0.00185311	0.00071461
Ctrl	**S08**	0.0241981	0.00286814	0.00061433	6.2577E-05	0.00021888	0.00052084	6.7447E-05	0.00106885	0.00108619	0.00037835	0.0010788	0.00109779	0.464777	0.00011667
Ctrl	**S09**	0.0607875	0.00519604	0.00095851	0.00019047	0.00014227	0.0021632	0.00179497	0.00365228	0.00290094	0.00288798	0.00219588	0.00190966	0.00129303	0.00208373
Ctrl	**S10**	0.0530479	0.00595327	0.00102628	0.00029978	0.00034266	0.00155552	0.00090885	0.00252158	0.00236119	0.00109332	0.00223033	0.0024288	0.00051738	0.00214582
Ctrl	**S11**	0.0432863	0.00499588	0.00089157	0.00013682	0.00036397	0.00108035	0.00072725	0.00078908	0.00129312	0.00054138	5.1195E-05	0.00137689	0.00240477	0.00052367
Ctrl	**S12**	0.0788965	0.00903065	0.00138995	0.00028877	0.00040642	0.00187535	0.00267765	0.00495653	0.0045104	0.00425731	0.00132404	0.0013879	0.00104941	0.00161107
GG2	**S01**	0.0528563	0.00513484	0.00120524	0.00014722	0.00019911	0.00247408	0.00206673	0.0030818	0.00288032	0.00163171	0.00384732	0.00420306	0.0022965	0.00250763
GG2	**S02**	0.031756	0.00438085	0.00041516	0.00021262	0.00014513	0.0006041	7.0677E-05	0.00064937	0.00111651	0.00029106	0.00053544	0.00142905	0.00035556	0.00043765
GG2	**S03**	0.0376186	0.00392976	0.00132798	0.00033531	0.00195955	0.00157328	0.00395088	0.00276272	0.003458	0.00213801	0.00434527	0.00383415	0.424564	0.0037635
GG2	**S04**	0.0252275	0.00480754	0.00150668	0.00036558	0.00025906	0.00120423	0.00110366	0.00230673	0.00113931	0.00115126	0.00222358	0.0015448	0.00307399	0.00155678
GG2	**S05**	0.0481041	0.00418359	0.00075497	6.9001E-05	0.00043147	0.000909	0.00197806	0.00162498	0.00168995	0.00073433	0.00249184	0.00213883	0.0055775	0.00090319
GG2	**S06**	0.022548	0.00373774	0.00064729	0.00011304	0.00017965	0.00034904	0.00089172	0.00059398	0.00059286	0.00145497	0.00075721	0.00011367	0.00307309	0.00030782
GG2	**S07**	0.0276112	0.00456184	0.00100637	0.00022401	0.00027783	0.00180036	0.00153612	0.00319586	0.00366134	0.00191381	0.0025956	0.00290862	0.00086757	0.00269314
GG2	**S08**	0.0334162	0.00249044	0.0005327	0.00022011	0.00028996	0.00596406	0.00019287	0.00127686	0.00099241	0.00078472	3.6288E-05	0.00107109	3.1155E-05	0.0029542
GG2	**S09**	0.0456506	0.00592601	0.00134883	0.00024862	0.00033242	0.00214376	0.00174603	0.00255419	0.00369518	0.00464702	0.001877	0.00213603	0.0044092	0.00477235
GG2	**S10**	0.0632677	0.00372459	0.00118073	0.00017389	0.00057016	0.0014246	0.00214468	0.00245035	0.00230061	0.00153006	0.00251255	0.00386818	0.00779165	0.00276549
GG2	**S11**	0.0209897	0.00301203	0.00040733	0.00017386	0.00022319	0.00042839	0.00016984	0.0002943	0.00030491	0.00017473	2.318E-05	0.00061444	0.00185203	6.7603E-05
GG2	**S12**	0.0631225	0.00547805	0.00111746	0.00035061	0.00015684	0.00174481	0.00187989	0.00355094	0.003362	0.00226594	0.00160613	0.00165873	0.00275417	0.00063827
GG4	**S01**	0.108865	0.00537761	0.0011309	0.00046307	0.00081809	0.00060365	0.0012851	0.00402703	0.00409072	0.0024666	0.00412457	0.00220272	0.00386024	0.00265048
GG4	**S02**	0.0213215	0.00286915	0.00045113	0.00010029	0.00030738	0.0004381	0.00029147	0.00079137	0.00057408	0.00019445	0.00018096	0.00022741	0.00025999	0.00048087
GG4	**S03**	0.0217949	0.00304545	0.00073096	0.00367369	0.00036181	0.00077964	0.00149259	0.001631	0.0025496	0.0006476	0.00146474	0.00284872	0.00081283	0.00113357
GG4	**S04**	0.0230384	0.00412267	0.00099924	7.7282E-05	0.00023231	0.00057336	0.00049947	0.00059535	0.0019478	0.00042836	0.00084505	0.0005532	0.00126505	0.00097704
GG4	**S05**	0.0670327	0.0042731	0.00112305	0.00581881	0.00055382	0.00194855	0.00343408	0.00262068	0.00437114	0.00628237	0.00468632	0.00335661	0.00320733	0.00340245
GG4	**S06**	0.021839	0.00248159	0.00042018	0.00010763	0.00019731	0.00059582	0.0002397	0.00071321	0.00036761	0.00046144	0.00065644	0.00023191	0.00145741	0.00014798
GG4	**S07**	0.0310165	0.00533275	0.00080353	0.00036018	0.00036258	0.00104998	0.00057665	0.00275989	0.00235137	0.00186314	0.00218499	0.0038362	0.00011986	0.00634524
GG4	**S08**	0.0252632	0.0046199	0.00071122	0.00014244	0.00031781	0.0029031	0.00190613	0.00180852	0.00210653	0.00096966	0.00023983	0.00242503	0.160922	0.00170818
GG4	**S09**	0.0379984	0.00482021	0.00095575	0.00014079	0.00026812	0.00159325	0.00024311	0.00302389	0.00304261	0.0033764	0.0031363	0.00072961	0.00162905	0.00182645
GG4	**S10**	0.0314977	0.00330763	0.00089354	0.00039307	0.00032729	0.00137093	0.00074484	0.00170924	0.00152888	0.00099249	0.00212019	0.00281125	0.00304704	0.00133004
GG4	**S11**	0.0167067	0.0021402	0.0004186	0.00014078	6.0493E-05	0.00063319	0.00028686	0.00022775	0.00022516	0.00012984	0.00012211	0.0893092	0.00095727	0.00023539
GG4	**S12**	0.0214161	0.00439013	0.00065387	0.00017925	0.0001368	0.00034202	6.7906E-05	0.00091628	0.00034144	0.00027464	0.00041258	0.00033964	0.00101247	0.139306

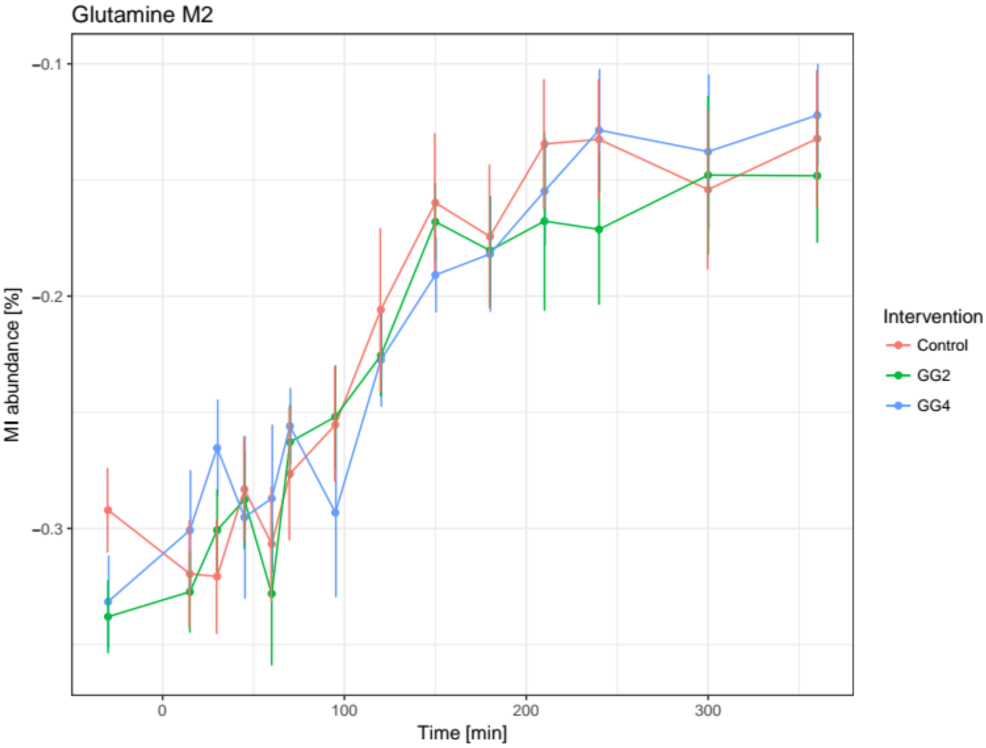
Supplementary Figure S1: Postprandial effect of wheat bread intake – Heatmap of significantly altered metabolites over time (Median of all subjects, Baseline correction, zScore Normalization)



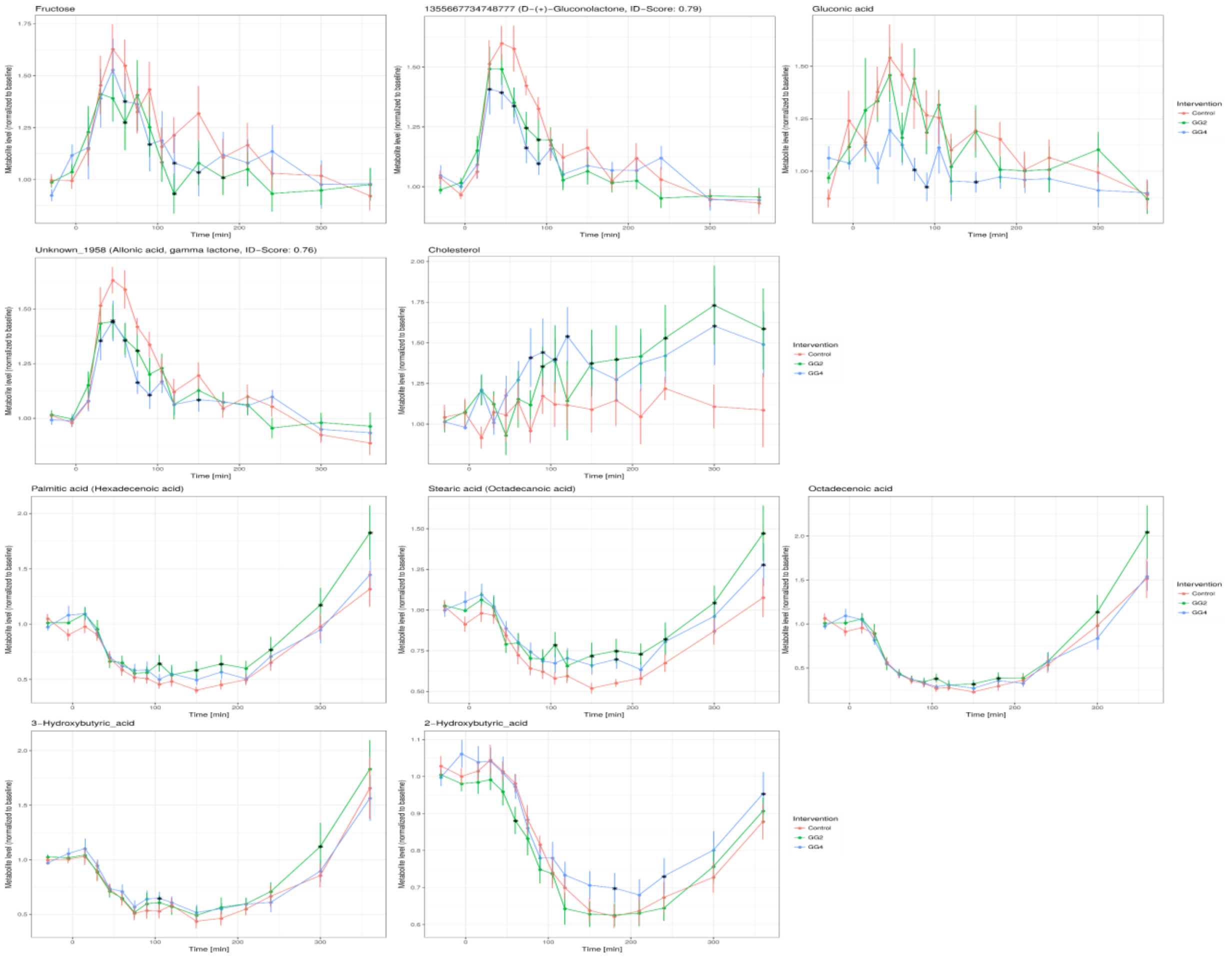
Supplementary Figure S2: Time-resolved metabolite levels after wheat bread intake – Line graphs of medians of all subjects with median average deviation



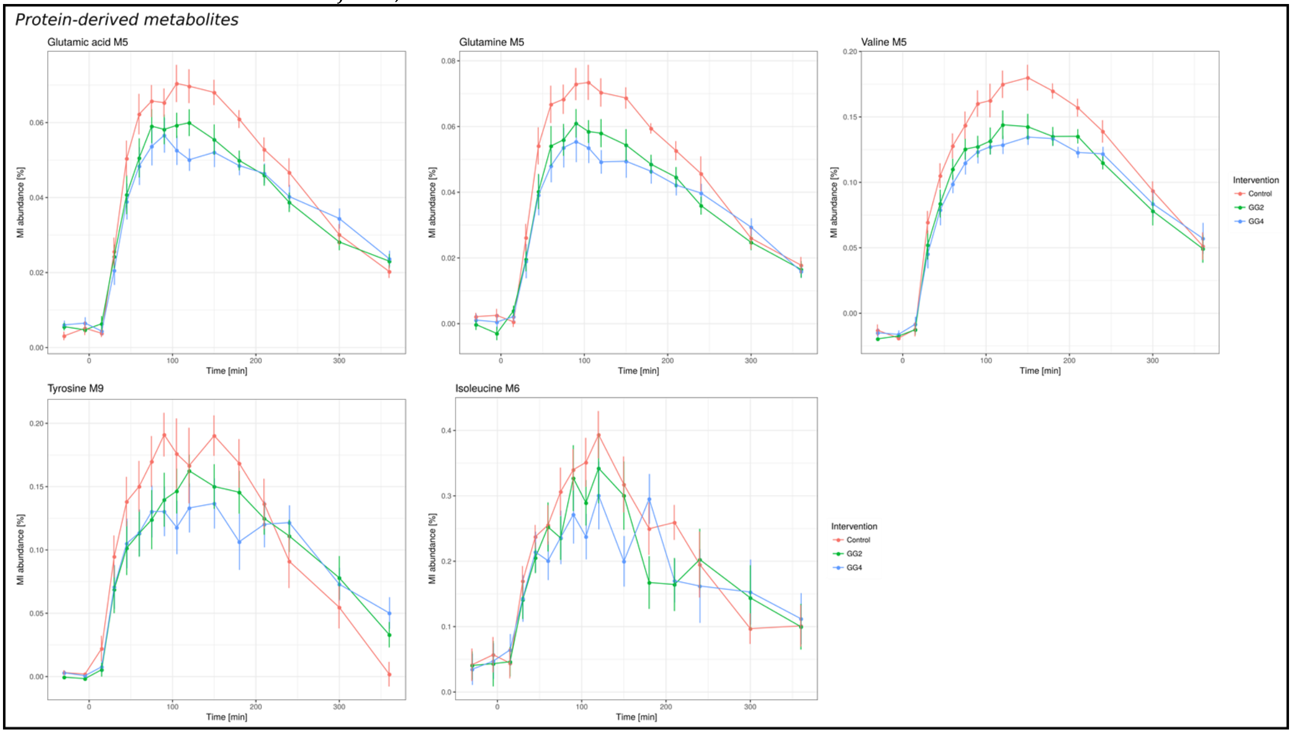
Supplementary Figure S3: ^{13}C-enrichment profile over time upon intervention for glutamine M2 – due to low enrichment, reliable calculations were omitted and negative values were obtained (Red – Control, Green – GG2, Blue – GG4; Average of MI abundance in % ± standard error of 12 subjects; upper box – starch-derived metabolites, lower box – metabolites of mixed origin)



Supplementary Figure S4: Significantly altered metabolite level profiles upon intervention – Fructose, 1355667734748777 (putative Gluconolactone), Gluconic acid, Unknown_1958 (putative Allonic acid gamma lactone), Cholesterol and palmitic acid, stearic acid, octadecenoic acid, 3-hydroxybutyric acid and 2-hydroxybutyric acid

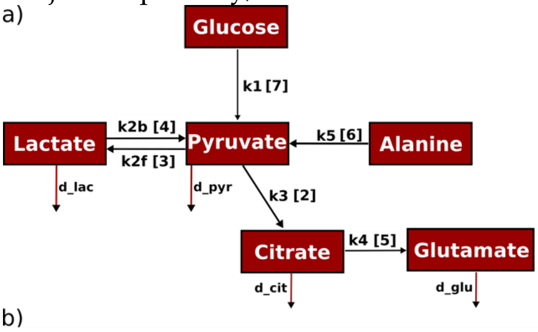


Supplementary Figure S5: Response curves of ^{13}C-enrichment profiles over time upon intervention for protein-derived metabolites – glutamic acid M5, glutamine M5, valine M5, tyrosine M9, isoleucine M6 (Red – Control, Green – GG2, Blue – GG4; Average of MI abundance in % ± standard error of 12 subjects)

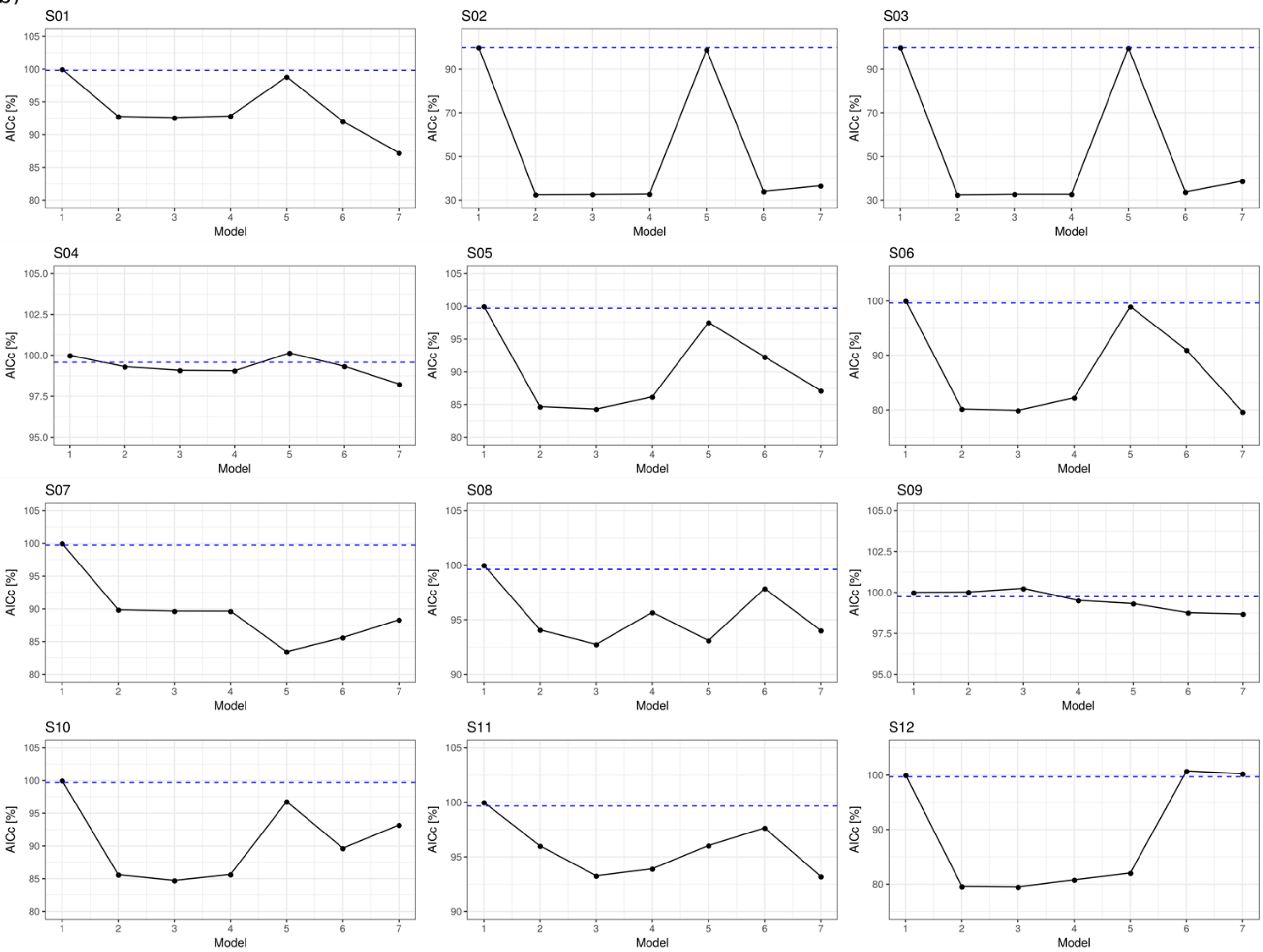


Supplementary Figure S6: Metabolic network model – a) Network model connecting the metabolites glucose, pyruvate, lactate, alanine, citrate and glutamate where model [1] represents the fixed rates model and [2]-[7] represent the flexible rates models where the number indicates the respective flexible rates (e.g. model [2] – rate k_3 flexible); b) AICcs calculated for the 7 different models (indexed in a) and normalized to the AICc obtained for Model 1 (all rates fixed) for all 12 subjects separately, blue dashed line indicates significantly decreased AICc compared to Model 1.

a)



b)



References

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[2] Boers, H.M., Van Dijk, T.H., Hiemstra, H., Hoogenraad, A.R., Mela, D.J., Peters, H.P.F., et al., 2017. Effect of fibre additions to flatbread flour mixes on glucose kinetics: A randomised controlled. British Journal of Nutrition 118(10): 777–87, Doi: 10.1017/S0007114517002781.