Supplementary information for

Magnesium stable isotope composition, but not concentration, responds to obesity and early insulin-resistant conditions in minipigs

Samuel le Goff, Jean-Philippe Godin, Emmanuelle Albalat, José Manuel Ramos Nieves, Vincent Balter

Corresponding author: Vincent.balter@ens-lyon1.fr

This file includes:

Supplementary Figures S1-S7

Supplementary Table S1-S5

Supplementary Reference S1

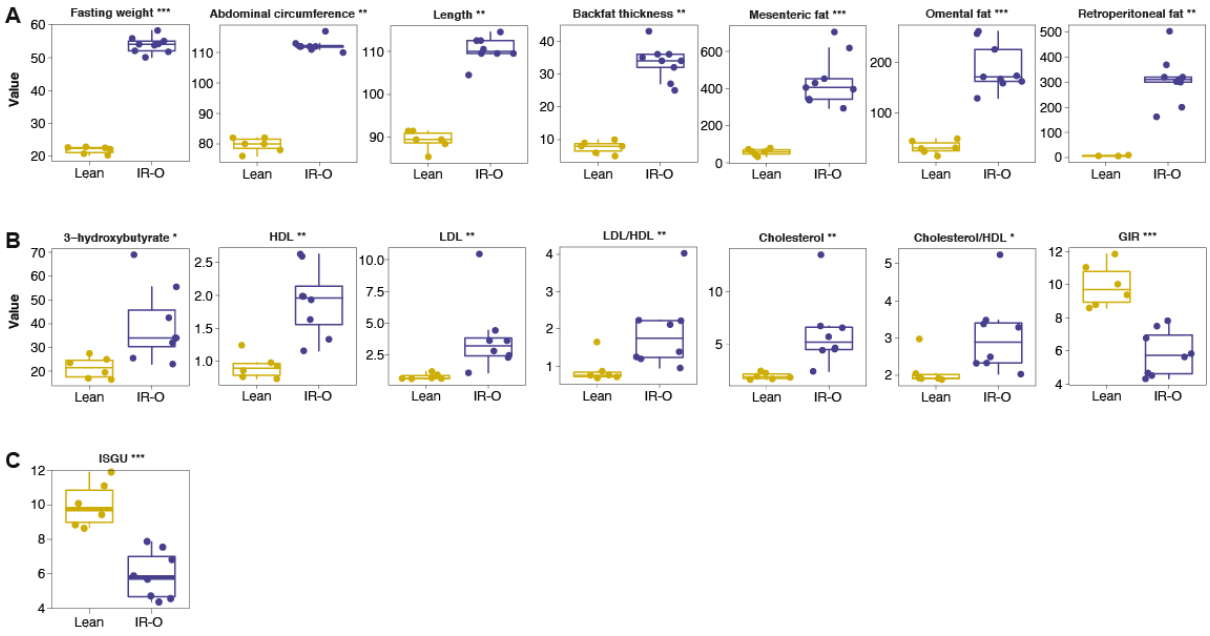

Figure S1: **Boxplots for significantly different phenotypes**. A) Clinical phenotypes. B) Molecular phenotypes. C) Clamp values. ISGU stands for insulin-stimulated systemic glucose utilization. Gold and dark blue stand for lean and IR-O minipigs, respectively. *P* values for Wilcoxon test are given: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

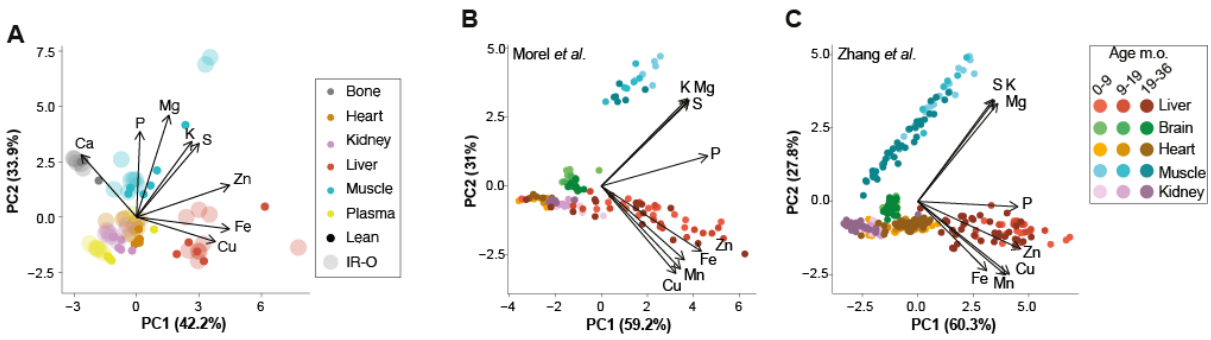

Figure S2: **PCA analysis of the metallome in the minipig and the mouse models**. A) PCA analysis of the present study in minipigs. B) PCA analysis in mice from Morel et al. (45). C) PCA analysis in mice from Zhang et al. (44). Data are normalized to Na for comparison.

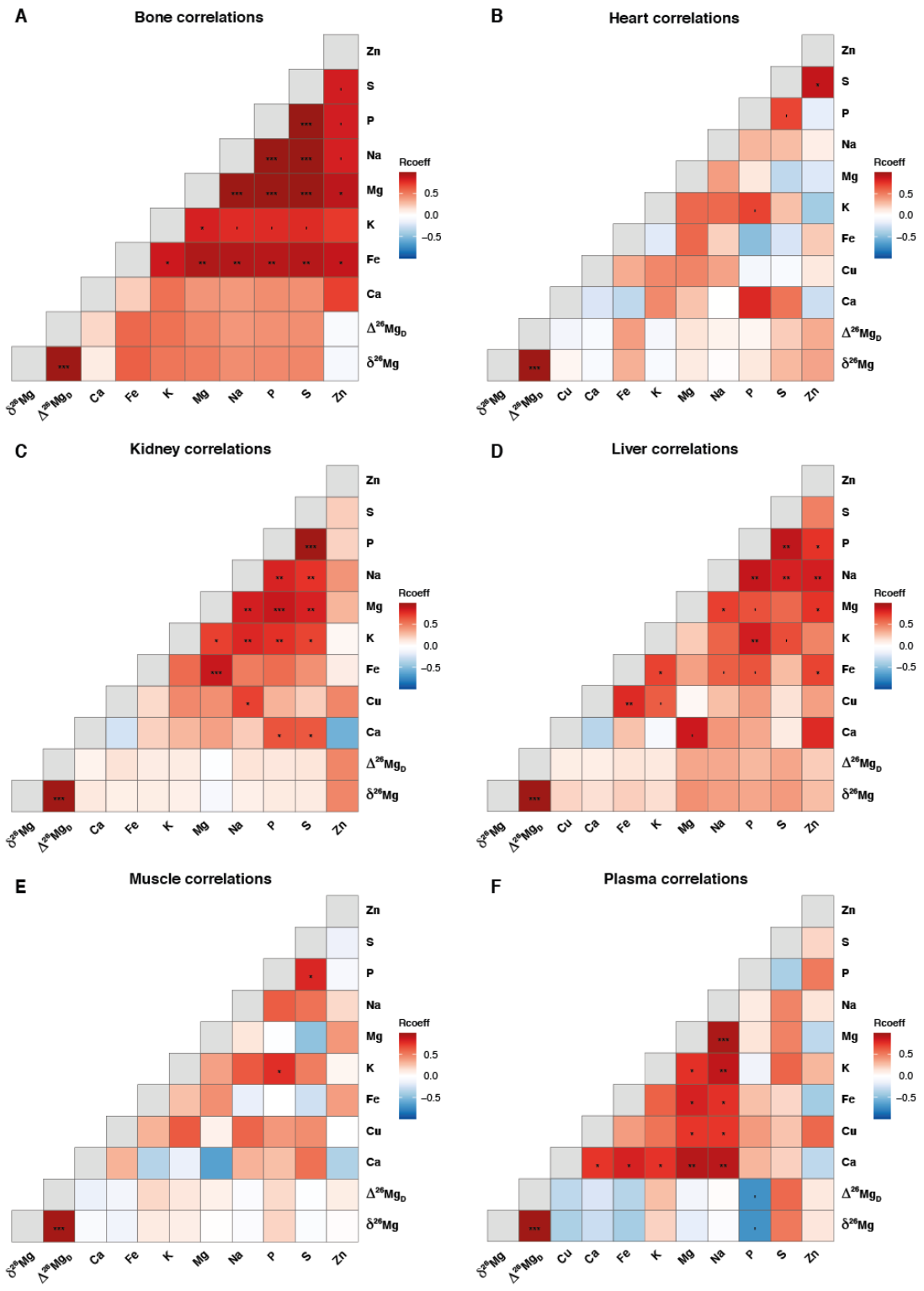

Figure S3: **Heatmap of Spearman Rho coefficients of correlations between the metallome and the Mg isotope compositions in organs**. Adjusted *P* value for multiple testing with the Benjamini & Hochberg correction are given: ***Adj.*P* < 0.001, **Adj.*P* < 0.01, *Adj.*P* < 0.05, ‘Adj.*P* < 0.1.

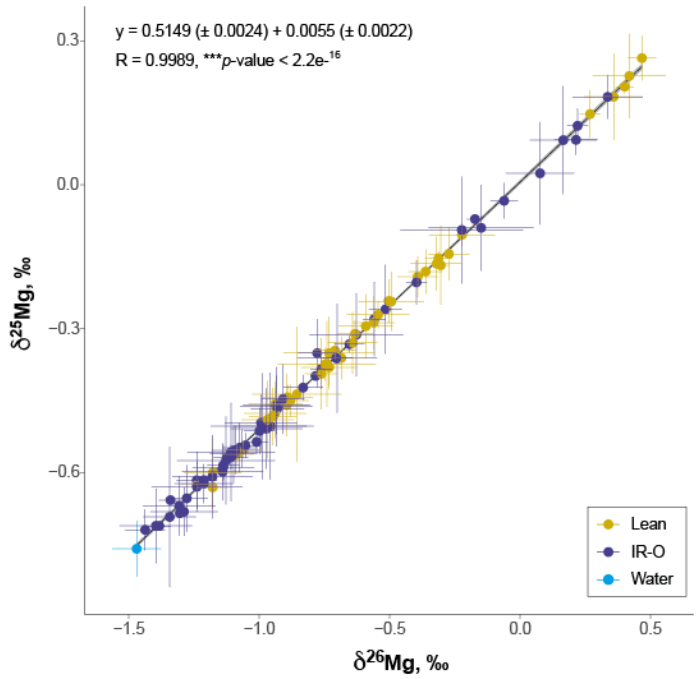

Figure S4: **Mass fractionation in three-isotope space of the results**. The slope of the regression line is 0.515, lying between the theoretical kinetically controlled (0.511) and thermodynamically controlled (0.521) values (ref S1)

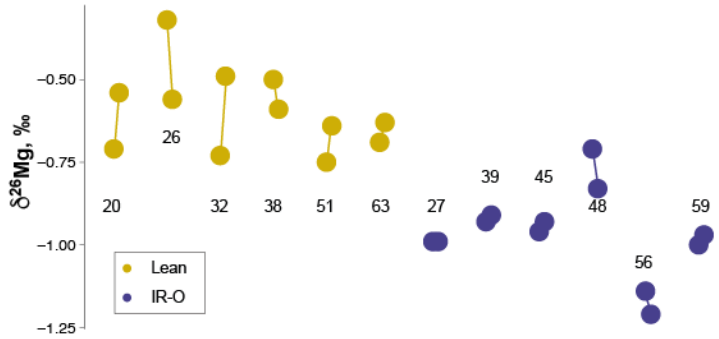

Figure S5: **Dot plot of the values of plasma**. The samples were collected 30 and 10 min before hyperinsulinemic-euglycemic clamp. ID of the animals are indicated.

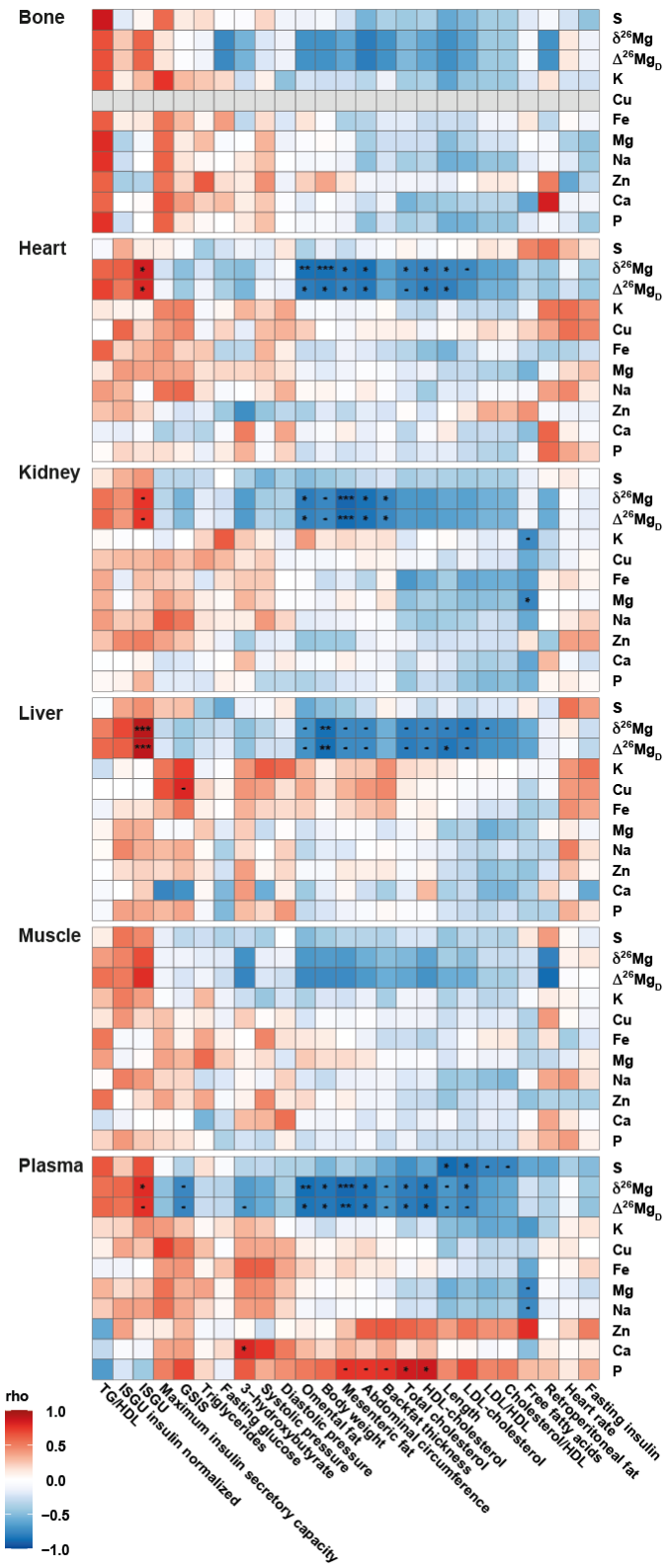

Figure S6: **Heatmap of Spearman Rho coefficients of correlations between the metallome and the Mg isotope compositions, and phenotypes in organs**. Adjusted *P* value for multiple testing with the Benjamini & Hochberg correction are given: ***Adj.*P* < 0.001, **Adj.*P* < 0.01, *Adj.*P* < 0.05, ‘Adj.*P* < 0.1.

Figure S7: **Scatterplots for significant correlations between the Δ26Mg$_D$ values and phenotypes**. Some correlations are spurious, notably concerning morphological phenotypes (e.g., fasting weight, length or abdominal circumference) because they are driven by only two groups of clustered values. Gold and dark blue stand for lean and IRO minipigs, respectively Spearman Rho coefficients and associated *P* value are given: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Table S1: **Phenotypes values of Göttingen minipigs**

Ind	Status	Abdominal circumference	Omental fat	Retroperitoneal fat	Backfat thickness	Mesenteric fat	Body length	Body weight	Systolic blood pressure	Diastolic blood pressure	Heart rate	Insulin-stimulated systemic glucose utilization (ISGU)	GIR insulin normalized	3-hydroxybutyrate	Total cholesterol	Total cholesterol:HDL ratio	Free fatty acids	Fasting glucose	Fasting insulin	HDL-cholesterol	LDL-cholesterol	LDL:HDL ratio	Triglycerides	TG:HDL ratio	Glucose-stimulated insulin secretory capacity (GSIS)	Maximum insulin secretory capacity
20	Lean	82	48	9.4	9	80	88	23	115	74	87	9.4	0.11	20	2.395	1.92	0.50	4.1	14.0	1.25	0.95	0.76	0.35	0.28	82	199
26	Lean	78	13		5	33	91	21	114	101	135	11.1	0.48	24	1.775	1.91	0.30	4.0	16.9	0.93	0.63	0.68	0.20	0.21	75	81
38	Lean	80	43	6.2	8	74	89	23	120	72	57	8.8	0.10	17	1.565	2.05	0.20	4.7	1.2	0.77	0.66	0.86	0.35	0.45	41	81
32	Lean	82	22		8	60	91	23	131	87	106	8.6	0.17	17	2.185	2.97	0.35	4.3	17.8	0.74	1.21	1.65	0.39	0.52	127	231
51	Lean	76	29		6	45	85	20	162	122	102	11.9	0.29	25	1.620	1.88	0.30	4.9	16.9	0.86	0.65	0.75	0.24	0.27	103	202
63	Lean	80	28	5.7	10	61	89	22	128	84	82	10.0	0.14	28	1.875	1.91	0.25	4.6	9.7	0.98	0.70	0.71	0.42	0.42	76	173
16	Obese		174	321	32	406		52																		
22	Obese	112	167	303	36	294	114	52	127	93	115	7.8	0.30	23	6.720	3.38	0.65	5.4	21.0	1.99	4.44	2.23	0.24	0.12	109	128
27	Obese	112	159	163	34	453	112	54	137	94	78	4.7	0.05	26	13.590	5.24	0.80	4.0		2.60	10.45	4.03	0.36	0.14		
34	Obese	112	162	370	27	430	109	54	114	77	75	5.8	0.11	32	6.555	2.49	0.35	5.0	12.8	2.63	3.64	1.38	0.40	0.15	71	98
39	Obese	110	258	321	25	338	112	55	142	113	106	4.3	0.08	34	4.390	3.29	0.45	5.0	15.6	1.34	2.82	2.11	0.28	0.21	102	133
45	Obese	113	226	311	35	618	109	56	181	128	85	5.6	0.18	56	5.690	3.48	0.25	4.5	7.7	1.64	3.63	2.22	0.43	0.26	148	245
48	Obese	111	129	504	43	343	104	50	154	108	113	6.8	0.09	69	2.355	2.03	0.35	4.6	20.2	1.16	1.10	0.95	0.20	0.17	159	321
56	Obese	117	263	301	34	704	109	58	134	94	108	4.5	0.07	34	4.490	2.32	0.30	5.2	19.5	1.94	2.31	1.19	0.45	0.23	166	228
59	Obese	112	171	201	36	397	110	54	139	89	109	7.5	0.10	43	4.620	2.33	0.30	4.8	24.5	1.99	2.48	1.25	0.24	0.12	134	216

Table S2: **Mg isotope composition and metallomic values of Göttingen minipigs**

Ind	Status	Organ	$\delta^{26}Mg$	±2SD	$\delta^{25}Mg$	±2SD	n	Ca	Cu	Fe	K	Mg	Na	P	S	Zn
20	Lean	Bone	-1.09	0.06	-0.56	0.02	3	1118		11	148	1992	2391	37781	580	273
26	Lean	Bone	-1.18	0.11	-0.60	0.07	3	1045		2	51	763	991	17237	262	26
32	Lean	Bone	-1.18	0.06	-0.63	0.04	3	1262		4	113	1155	1292	21920	334	41
22	Obese	Bone	-1.38	0.04	-0.71	0.01	2	897		3	67	734	941	15361	233	29
27	Obese	Bone	-1.24	0.08	-0.62	0.06	2	881		2	50	784	979	15896	244	30
34	Obese	Bone	-1.40	0.14	-0.71	0.08	2	1176		2	79	910	1198	19834	304	35
39	Obese	Bone	-1.34	0.04	-0.66	0.01	2	1127		4	69	1446	1793	29145	443	41
48	Obese	Bone	-1.34	0.10	-0.69	0.15	2	1196		3	83	1030	1348	22037	330	33
20	Lean	Heart	-0.39	0.08	-0.19	0.04	5	14	27	333	13731	1037	3673	8964	9057	111
26	Lean	Heart	-0.27	0.08	-0.14	0.05	5	1036	24	257	15750	1230	3709	11840	9653	
32	Lean	Heart	-0.36	0.06	-0.18	0.05	4	19	28	333	16558	1230	4930	10971	10195	119
38	Lean	Heart	-0.31	0.05	-0.15	0.01	3	97	18	338	11796	1017	3490	8947	8196	87
51	Lean	Heart	-0.30	0.03	-0.17	0.08	2		28	394	15445	1381	3603	9920	8271	33
63	Lean	Heart	-0.22	0.13	-0.10	0.02	2	21	23	285	15719	1263	3564	10080	7411	15
16	Obese	Heart	-0.70	0.15	-0.36	0.11	2		36	526	16099	2518	3685	9954	8007	1722
22	Obese	Heart	-0.56	0.04	-0.28	0.08	5	17	28	284	14096	1063	3614	9404	9380	107
27	Obese	Heart	-0.63	0.18	-0.31	0.09	3	94	19	323	12439	975	2324	8770	8243	84
34	Obese	Heart	-0.76	0.04	-0.38	0.04	5	609	19	221	15073	1163	3487	12022	10135	
39	Obese	Heart	-0.65	0.06	-0.33	0.02	3	96	9	161	5583	470	923	4312	4079	43
45	Obese	Heart	-0.52	0.06	-0.26	0.09	2		28	367	14983	1255	3986	9511	8788	67
48	Obese	Heart	-0.40	0.04	-0.20	0.05	5	698	23	291	17200	1038	3773	12948	10616	
56	Obese	Heart	-1.07	0.00	-0.55	0.04	2		24	318	15917	1258	4502	9800	7403	12
59	Obese	Heart	-0.78	0.02	-0.40	0.00	2		26	250	15293	1289	3084	9674	7521	25
20	Lean	Kidney	-0.88	0.07	-0.45	0.03	5	23	63	254	10524	846	5025	10401	7592	119
26	Lean	Kidney	-0.76	0.06	-0.39	0.07	5	1179	46	282	11225	960	6873	16144	10171	
32	Lean	Kidney	-0.73	0.11	-0.38	0.04	5	26	103	261	9942	933	8543	12154	8393	149
38	Lean	Kidney	-0.90	0.08	-0.46	0.07	3	977	66	338	14498	1186	7607	16638	11170	30
51	Lean	Kidney	-0.86	0.17	-0.44	0.14	2	177	139	294	11574	1019	9149	12138	7507	88
63	Lean	Kidney	-0.74	0.19	-0.37	0.09	3	141	192	334	12210	1077	7598	12305	7501	131
16	Obese	Kidney	-1.14	0.06	-0.58	0.04	2	174	56	363	11989	1057	6003	11591	7387	65
22	Obese	Kidney	-1.10	0.09	-0.56	0.06	5	24	87	224	11617	826	6476	11762	8705	128
27	Obese	Kidney	-1.11	0.17	-0.56	0.10	3	105	29	172	3007	329	1274	4099	3299	45
34	Obese	Kidney	-1.08	0.07	-0.55	0.05	5	726	89	200	11563	887	6745	15009	9987	38
39	Obese	Kidney	-0.93	0.10	-0.47	0.02	2	84	14	49	1451	189	838	2484	1785	29
45	Obese	Kidney	-1.30		-0.68		1	255	136	258	12180	1068	8225	11596	7482	41
48	Obese	Kidney	-1.11	0.07	-0.57	0.03	5	608	61	416	11100	1611	7773	16542	10532	410
56	Obese	Kidney	-1.14	0.16	-0.60	0.06	2	307	126	297	12455	1125	8485	12334	7550	108
59	Obese	Kidney	-1.01	0.04	-0.54	0.02	2	145	161	325	13116	1036	8007	12382	7382	86
26	Lean	Liver	0.47	0.05	0.26	0.05	4	442	28	1465	8770	2310	3744	14240	8723	801
32	Lean	Liver	0.27	0.04	0.15	0.05	4	17	35	818	7674	699	2591	10592	7720	268
38	Lean	Liver	0.42	0.14	0.23	0.09	2	94	19	767	4791	424	598	6770	4708	172
51	Lean	Liver	0.36	0.11	0.18	0.09	3		31	969	10399	817	2253	11374	6618	391
63	Lean	Liver	0.40	0.03	0.20	0.01	2		47	1708	8514	1258	3555	12013	7041	893
16	Obese	Liver	0.34	0.13	0.18	0.05	2		84	1704	9258	814	1244	9969	5196	400
22	Obese	Liver	0.21	0.08	0.09	0.03	4	15	38	1362	9087	602	2365	10280	7005	317
27	Obese	Liver	-0.22	0.24	-0.09	0.11	3	104	23	844	9097	636	1388	10850	6937	363
34	Obese	Liver	-0.06	0.05	-0.03	0.04	3	322	17	621	4274	955	1329	6959	3884	345
39	Obese	Liver	-0.17	0.03	-0.07	0.01	2	91	18	749	4801	376	781	6600	4121	147
45	Obese	Liver	0.17	0.13	0.09	0.11	2		51	1472	9750	832	2821	12252	6619	654
48	Obese	Liver	0.22	0.04	0.12	0.04	4	52166	46	875	33921	7656	9786	30878	8247	403
56	Obese	Liver	-0.15	0.20	-0.09	0.09	2		61	1740	9332	846	2578	10680	6015	664
59	Obese	Liver	0.08	0.13	0.02	0.11	2		54	1740	10992	779	2607	12167	6673	430
20	Lean	Muscle	-1.24	0.06	-0.62	0.03	3	17	8	79	15286	1143	1942	8614	9845	195
26	Lean	Muscle	-0.95	0.09	-0.48	0.06	5	506	14	67	16169	901	2036	9308	10854	105
32	Lean	Muscle	-0.89	0.10	-0.44	0.03	4	13	14	192	17160	1169	1556	8684	8514	124
38	Lean	Muscle	-0.94	0.12	-0.48	0.05	2	97	5	109	12365	965	738	6974	8027	175
51	Lean	Muscle	-0.94	0.10	-0.46	0.05	2	132	13	137	15077	1155	1865	7457	8745	244
63	Lean	Muscle	-1.06	0.07	-0.55	0.02	2	3	9	356	15564	1325	1334	7431	7572	179
16	Obese	Muscle	-1.29	0.13	-0.68	0.05	3	68	9	150	15504	1256	1787	8161	8469	239
22	Obese	Muscle	-1.05	0.05	-0.54	0.03	5	12	5	32	14275	943	1661	7489	8040	106
27	Obese	Muscle	-1.13	0.19	-0.57	0.09	3	99	4	94	12937	965	515	7336	7751	132
34	Obese	Muscle	-1.44	0.08	-0.72	0.04	5	810	18	102	16639	1016	1692	10384	10346	89
39	Obese	Muscle	-1.31	0.14	-0.67	0.06	3	92	6	133	12594	967	490	7019	7347	133
45	Obese	Muscle	-1.24	0.17	-0.63	0.05	3	73	15	127	14994	1071	1816	7678	8492	283
48	Obese	Muscle	-1.28	0.08	-0.65	0.07	5	677	12	76	13042	737	1941	7688	9262	111
56	Obese	Muscle	-1.21	0.04	-0.62	0.00	2	62	8	98	15179	1429	1736	7956	7536	170
59	Obese	Muscle	-1.18	0.15	-0.61	0.09	4	72	14	118	13697	1347	1506	6916	6291	139

Table S2: **continued**

Ind	Status	Organ	$\delta^{26}Mg$	±2SD	$\delta^{25}Mg$	±2SD	n	Ca	Cu	Fe	K	Mg	Na	P	S	Zn
20	Lean	plasma-1	-0.71	0.08	-0.35	0.02	5	51	38	17	19820	210	32559	1329	10072	13
20	Lean	plasma-2	-0.54	0.12	-0.27	0.03	3	46	38	18	19744	214	34044	1364	10043	14
26	Lean	plasma-1	-0.32	0.10	-0.16	0.06	4									
26	Lean	plasma-2	-0.56	0.08	-0.29	0.06	3									
32	Lean	plasma-1	-0.73	0.17	-0.35	0.08	3	50	34	28	19515	200	34468	1094	9491	13
32	Lean	plasma-2	-0.49	0.13	-0.24	0.06	3	47	32	25	20288	196	34924	1135	9529	14
38	Lean	plasma-1	-0.50	0.02	-0.24	0.05	3	58	28	38	20253	260	37028	1078	11255	13
38	Lean	plasma-2	-0.59	0.10	-0.30	0.07	3	53	25	37	19669	237	33829	945	10170	13
51	Lean	plasma-1	-0.75	0.06	-0.37	0.03	4	1349	46	46	23394	318	40641	1219	10191	
51	Lean	plasma-2	-0.64	0.09	-0.33	0.06	5	1384	44	44	23086	311	42520	1258	10302	
63	Lean	plasma-1	-0.69	0.09	-0.36	0.04	3	1280	34	42	22592	282	38989	1277	9895	
63	Lean	plasma-2	-0.63	0.07	-0.31	0.03	2	1387	35	40	22692	293	41189	1267	9747	
22	Obese	plasma-1	-0.78	0.07	-0.35	0.07	3	51	32	19	17764	165	31043	1396	9353	14
27	Obese	plasma-1	-0.99	0.20	-0.50	0.11	2	70	34	34	16381	190	30996	2036	9213	14
27	Obese	plasma-2	-0.99	0.14	-0.50	0.06	2	49	27	34	16180	185	29818	1966	8929	14
34	Obese	plasma-1	-1.12	0.01	-0.57	0.02	2									
39	Obese	plasma-1	-0.93	0.14	-0.46	0.08	3	57	24	27	13775	176	27246	1019	7691	11
39	Obese	plasma-2	-0.91	0.08	-0.45	0.07	3	56	29	35	17945	211	32491	1237	9851	11
45	Obese	plasma-1	-0.96	0.14	-0.50	0.11	4	1376	44	37	19757	296	41435	1826	9494	
45	Obese	plasma-2	-0.93	0.13	-0.46	0.06	3	1357	52	47	20208	340	42032	1827	9680	
48	Obese	plasma-1	-0.71	0.06	-0.36	0.01	2									
48	Obese	plasma-2	-0.83	0.08	-0.42	0.03	2									
56	Obese	plasma-1	-1.14	0.12	-0.59	0.03	5	1288	43	45	20162	307	37801	1573	9836	
56	Obese	plasma-2	-1.21	0.11	-0.62	0.04	6	1300	41	41	20400	273	37732	1572	9892	
59	Obese	plasma-1	-1.00	0.06	-0.51	0.05	3	1421	37	57	23736	284	41266	1672	9676	
59	Obese	plasma-2	-0.97	0.14	-0.51	0.08	3	1390	36	46	23152	278	40543	1604	9325	

Table S3: **Calculation of the whole diet Mg isotope composition**. The calculation includes Mg isotope composition and concentration in solid food and water and assumes that the water/food mass ratio for Göttingen pigs is 2.5.

Mean feed intake pellets (g/day/cage/2 pigs)

			Days																						
Cage	Batch nr	Treatment	73-86	87-100	101-114	115-128	129-142	143-156	157-170	171-184	185-198	199-212	213-226	227-240	241-254	255-268	269-282	283-296	297-310	311-324	325-331	Total food intake (kg)	Total water intake(kg)	Total food Mg intake (g) *	Total water Mg intake (g) #	δ^{26}Mg intake (‰) *#¥
10	1	Obese	500	600	943	1000	1000	1000	1000	1000	1343	1400	1400	1400	1400	1400	1500	1600	1600	1600	1600	11.6	29.1	19.9	1.3	-1.08
15	2	Obese	500	600	829	1000	1000	1000	1000	1000	1343	1400	1400	1400	1400	1400	1500	1600	1600	1600	1600	11.6	29.0	19.8	1.3	-1.08
20	2	Obese	500	600	829	1000	1000	1000	1000	1000	1343	1400	1400	1400	1400	1400	1500	1600	1600	1600	1600	11.6	29.0	19.8	1.3	-1.08
25	3	Obese	500	600	914	1000	1000	1000	1000	1000	1343	1400	1400	1400	1400	1400	1500	1600	1600	1600	1600	11.6	29.1	19.9	1.3	-1.08
28	3	Obese	750	900	1371	1500	1500	1500	1500	1321	1343	1400	1400	1400	1400	1400	1500	1600	1600	1600	1600	13.3	33.2	22.7	1.5	-1.08
5	1	Obese	500	600	943	1000	1000	1000	1000	1000	1343	1400	1400	1400	1400	1400	1500	1600	1600	1600	1600	11.6	29.1	19.9	1.3	-1.08
3	1	Lean	400	400	400	457	500	600	600	600	686	700	700	700	750	800	850	900	900	900	900	6.4	15.9	15.2	0.7	-0.96
12	2	Lean	400	400	400	457	500	600	600	600	686	700	700	700	750	800	850	900	900	900	900	6.4	15.9	15.2	0.7	-0.96
19	2	Lean	400	400	400	457	500	600	600	600	686	700	700	700	750	800	850	900	900	900	900	6.4	15.9	15.2	0.7	-0.96
32	3	Lean	600	600	600	675	750	900	900	793	686	700	700	700	750	800	850	900	900	900	900	7.3	18.3	17.4	0.8	-0.96

* with a concentration of 2381 ppm and 1710 ppm for normal and semi-western diets, respectively.

with a concentration of 44 ppm in water.

¥ with a δ^{26}Mg value of -0.97‰, -1.09‰ and -1.44‰ for normal diet, semi-western diet and water, repectively.

Table S4: **Diet formulation of standard milk replacer minipig diet.**

Ingredient	Control milk replacer (Lactose)
WPC-75%CP	29.40%
Lactose	24.05%
Vana Grasa 80C (coconut/malto)	18.28%
Vana-Grasa 80D (palmoil/whey)	18.23%
Maltodextrin 20 M500	2.45%
Di-Potassium phosphate 17.7%P	1.47%
Citric acid Anh West	0.98%
Sodium chloride	0.98%
Calcium carbonate Pwd FineWhite	0.74%
SLOTEN 1942 PREMIX	0.49%
Calcium formate 98% Fine	0.49%
Calcium acetate 99% OAB	0.49%
Magnesium sulphate Anh 20%	0.49%
Emulsifier E487 silica	0.34%
Potassium sorbate	0.33%
DL-methionine 99% West	0.31%
L-Lysine HCl 98%	0.18%
L-Threonine 98%	0.08%
Silica Tix-O-Sil 38	0.06%
L-Tryptophan 98%	0.06%
Sweetener	0.03%
Vanilla Aroma	0.03%
Vit E 50% Adsorbate	0.02%
Iron sulphate 1aq 30% min Fine	0.02%
Copper sulphate 5aq 25% Fine	0.01%
	0.00%
Total	100%

Table S5: **Diet formulation of high-energy, obesogenic diet (SK2_2) and control, lean SDS minipig diet.**

	SK2_2 0.5% cholesterol	**SDS SMP(E) Standard Mini Pig Diet**
	%	
Barley	10.25	*
Wheat	8.00	*
Soja hulls RC 320-360	35.93	*
Potato protein	3.69	
Wheat gluten protein	5.16	
Sucrose	20.00	
Lard	8.50	
Trans-fat soja oil	4.00	
Cholesterol	0.50	
Limestone, CaCO3 (powder)	1.35	
Mono-Calcium phosphate	1.79	
NaCl	0.62	
Premix 2 g/kg	0.20	
L-Tryptophan	0.01	
Total	**100.00**	
Oat hulls and bran		*
Wheatfeed		*
De-hulled extracted toasted soya		*
Molasses		*
Sunflower extracted		*
Macro minerals		*
Micro minerals		*
Vitamins		*

Table S6: **Nutrient composition of the high-energy, obesogenic diet (OB) and control, lean SDS Minipig diet**

		OB 0.5% cholesterol	SDS SMP(E) Standard Mini Pig Diet
Dry matter	g	925.7	900
Crude ash	g	58.9	73.5
Crude protein	g	130.0	130.3
Crude fat	g	137.6	21.3
C18:2	g	12.5	3.1
Crude fiber	g	127.6	145.2
Digestible crude oil	g		12.0
Digestible crude protein	g		94.1
Starch (amylase method)	g	102.4	271.2
Sugars	g	222.9	55.4
Non-starch polysaccharides	g	279.8	
Nitrogen free extract	g		524.1
Total dietary fibre	g		296.6
EW	-	1.10	
Gross energy	MJ		13.8
Digestible energy	MJ		11.43
Metabolisable energy	MJ		10.98
Net energy diet	MJ	9.70	
Calcium	g	10.1	10.1
Phosphorus	g	5.3	5.9
Digestible phosphorus (faeces)	g	3.7	3.7
Inositol-phosphate	g	0.6	
Ca/digestible phosphorus		2.7	
Magnesium	g	1.1	3.1
Sodium	g	2.6	2.9
Potassium	g	6.0	13.7
Chloride	g	4.2	4.2
Electrolyte balance	meq	150	
Iron	mg	376	161.59
Copper	mg	25	17.73
Zinc	mg	94	118.08
Cobalt	µg		59.60
Iodine	µg		62.68
Selenium	µg		211.35
Fluorine	mg		16.58
LYS	g	6.3	6.3
MET	g	2.2	1.8
CYS	g	2.5	2.1
M+C	g	4.7	3.9
THR	g	4.9	4.9
TRP	g	1.6	1.6
ILE	g	5.4	5.3
ARG	g	6.1	8.8
PHE	g	6.7	6.2
HIS	g	3.0	3.3
LEU	g	9.9	9.2
TYR	g	5.5	3.7
VAL	g	6.4	6.4
ALA	g	5.1	0.6
ASP	g	10.1	5.07
GLU	g	27.2	18.1
GLY	g	6.7	10.0
PRO	g	10.6	7.4
SER	g	6.7	4.3
Cumulative AA	g	126.8	
Ileal digestible LYS	g	4.6	
Ileal digestible MET	g	1.9	
Ileal digestible CYS	g	1.9	
Ileal digestible M+C	g	3.7	
Ileal digestible THR	g	3.6	
Ileal digestible TRP	g	1.2	
Ileal digestible ILE	g	4.4	
Ileal digestible ARG	g	5.3	
Ileal digestible PHE	g	5.7	
Ileal digestible HIS	g	2.3	
Ileal digestible LEU	g	8.3	
Ileal digestible TYR	g	4.4	
Ileal digestible VAL	g	5.0	

Supplementary reference:

S1 E. D. Young, A. Galy, H. Nagahara, Kinetic and equilibrium mass-dependent isotope fractionation laws in nature and their geochemical and cosmochemical significance. *Geochim Cosmochim Acta*. **66**, 1095-1104 (2002).