Compound	Peak area of metabolites / peak area of IS					
	Fillet of Australian beef					
	0.02g		0.05g		0.1g	
	Average	CV (%)	Average	CV (%)	Average	CV (%)
Pyruvic acid	0.10	16.4	0.26	11.9	0.58	6.7
Lactic acid	87.42	1.1	127.31	3.9	183.81	4.7
Glycolic acid	0.05	5.4	0.15	19.0	0.39	9.0
Alanine	5.85	11.2	16.98	10.8	30.42	13.9
3-Hydroxybutyric acid	0.32	0.5	0.79	6.7	1.55	7.8
Valine	17.81	2.8	45.08	9.1	87.09	7.7
Urea	48.99	3.1	131.22	2.7	256.49	4.7
4-Hydroxybutyric acid	0.34	6.6	0.93	2.9	1.68	5.5
Dihydroxyacetone	0.05	7.0	0.13	11.4	0.26	7.6
Leucine	5.59	6.4	12.95	3.3	22.18	5.0
Glycerol	36.11	3.1	93.87	6.3	174.03	3.9
Phosphoric acid	200.51	3.2	292.69	2.9	397.38	4.6
Isoleucine	3.16	9.5	8.15	2.4	14.56	3.9
Proline	1.87	10.0	4.55	0.6	7.24	5.9
Succinic acid	0.97	5.5	2.59	8.0	4.83	7.2
Glycine	23.46	3.3	60.74	7.9	109.97	7.5
Glyceric acid	3.40	3.6	8.75	7.0	17.01	6.6
Fumaric acid	21.40	14.3	50.68	14.2	102.31	13.0
Uracil	0.12	8.5	0.36	16.0	0.75	7.7
Serine	2.51	6.5	6.65	4.5	12.11	6.1
Threonine	8.04	8.4	21.45	2.9	40.92	5.3
Glutaric acid	0.03	26.2	0.05	5.9	0.09	11.4
3-Aminopropanoic acid	0.62	1.8	1.67	9.6	3.10	6.7
Niacinamide	4.85	3.0	13.51	6.6	28.36	5.2
Malic acid	5.93	8.8	17.32	7.6	30.06	6.1
N-Acetylserine	0.16	5.7	0.51	7.7	0.98	3.1
Aspartic acid	0.19	7.0	0.35	2.5	0.64	3.4
Methionine	1.78	8.2	3.82	3.4	7.18	8.8
5-Oxoproline	2.89	4.9	8.33	5.5	18.39	18.7
4-Aminobutyric acid	0.17	11.4	0.46	8.7	0.92	8.3
Cysteine	10.15	5.5	20.69	2.4	35.61	6.9
Creatinine	69.33	2.9	84.36	5.4	63.42	6.4
Threonic acid	0.17	11.6	0.45	11.4	0.88	10.6
Hypotaurine	1.51	4.0	2.85	69.1	7.74	7.6
3-Aminoglutaric acid	0.35	0.5	0.97	11.4	1.79	8.7
Ornithine	3.16	8.4	5.95	5.5	7.89	7.2
Glutamic acid	0.33	9.4	0.86	5.0	1.47	9.2
5-Aminovaleric acid	0.03	16.9	0.06	15.3	0.13	7.0
Phenylalanine	34.33	4.4	86.03	5.5	159.06	5.9
Arabinose	1.28	45.4	3.10	75.6	5.73	18.7
Homocysteine	0.39	7.7	1.05	4.7	1.59	10.4
Asparagine	0.47	10.1	1.20	2.2	1.04	29.3
Ribose	83.75	5.3	222.07	5.0	402.91	5.4
Ribonolactone	0.02	173.2	0.15	19.2	0.30	5.5
Xylitol	1.76	3.6	1.88	115.2	8.99	7.1
Arabitol	0.63	12.2	1.00	3.5	1.76	3.1
Putrescine	0.81	3.5	1.97	10.4	3.60	8.3
Glycerol 3-phosphate	0.45	4.6	1.35	8.4	2.47	6.7
Glutamine	2.24	3.0	5.59	3.4	5.69	45.1
O-Phosphoethanolamine	0.13	7.5	0.39	11.8	0.71	11.2
Hypoxanthine	38.19	7.3	110.10	3.0	203.20	5.2
Galactose	1.07	38.9	2.66	81.2	5.68	22.0
Arginine	1.18	7.8	3.18	6.4	4.71	30.3
Citric acid + Isocitric acid	0.98	6.2	2.78	14.0	5.05	6.9
Psicose	88.04	24.2	252.84	3.7	388.16	6.4
Fructose	68.25	6.3	178.79	2.9	309.28	5.7
Allose	129.73	4.4	323.86	5.8	524.71	7.6
Mannose	315.20	50.8	631.13	4.1	779.81	3.8
Glucose	22.92	3.7	60.68	6.4	110.25	6.1
Lysine	26.53	5.1	63.78	8.3	98.56	5.6
Tyrosine	1.31	5.6	3.22	7.5	5.25	9.5
Mannitol	4.47	3.7	11.68	7.8	21.20	8.2
Pantothenic acid	0.19	3.1	0.53	5.7	1.03	7.2
Xanthine	3.96	3.9	11.61	5.8	21.36	5.4
Gluconic acid	2.69	5.0	7.43	7.4	13.68	6.9
Dopamine	0.30	10.6	0.86	14.3	1.64	8.0
N-Acetylmannosamine	0.05	66.5	0.25	10.8	0.48	7.7
Uric acid	0.10	1.2	0.32	0.8	0.36	18.0
Inositol	19.59	4.3	51.70	11.3	96.37	7.0
Ribulose 5-phosphate	0.47	9.1	1.27	8.1	2.49	7.2
Ribose 5-phosphate	0.03	89.5	0.12	36.8	0.23	26.7
Cystamine	0.12	26.1	0.94	22.2	2.32	6.5
Tryptophan	0.09	11.3	0.21	16.9	0.23	13.5
Fructose 1-phosphate	48.53	7.5	132.15	4.2	235.39	8.0
5-Methoxytryptamine	1.23	9.4	2.04	7.5	1.70	7.9
Inosine	7.23	0.8	18.10	4.3	30.06	6.0
Sedoheptulose 7-phosphate	0.76	26.1	1.95	7.6	3.03	2.6
Adenosine	N.D.	-	N.D.	-	0.21	12.7
Xanthosine	0.02	173.2	0.11	41.3	0.10	18.3
Trehalose	3.66	3.1	9.42	1.4	14.72	6.1
Maltose	0.58	4.6	1.54	5.7	2.52	11.4
Maltitol	N.D.	-	0.18	11.0	0.28	30.2
Isomaltose	0.16	3.0	0.38	9.8	0.71	7.6

Fig. S1

Table S1. Metabolites detected in lean beef by GC/MS

The reproducibility of GC/MS analysis was evaluated by CV%. The test was repeated three times using different amounts of muscle tissue (0.02, 0.05, or 0.1 g). For lean beef samples, we used the psoas major of Australian beef (commercial quality in Japan), which is primarily composed of slow-type muscle. The values colored with a white-red gradient indicate the relative deviation of the compound between analyses (light pink, CV > 20 %; red, CV > 30% or not detected). Over 80 metabolites were detected across the samples (20-mg sample, 81 metabolites; 50-mg sample, 82 metabolites; 100-mg sample, 83 metabolites), and the detection of most metabolites was confirmed to be concentration-dependent (20-mg sample, 70 metabolites; 50-mg sample, 75 metabolites; 100-mg sample, 77 metabolites).

Fig. S1