Supplementary Table 1. Statistics for energy intakes after treatment, related to Figure 1
2 x 2 - Mixed ANOVA

DGAT-1i dose	Time after food access (h)	Test of Within- Subjects Effects		Test of Between- Subject Effects
		Treatment:	Treatment x Diet:	Diet:
3 mg/kg	1	$F(1,7) = .011$; $P = .921$	$F(1,7) = 2.581$; $P = .152$	$F(1,7) = .370$; $P = .562$
	3	$F(1,7) = .011$; $P = .921$	$F(1,7) = .218$; $P = .655$	$F(1,7) = .000$; $P = .999$
	5	$F(1,7) = 4.842$; $P = .064$	$F(1,7) = 2.926$; $P = .131$	$F(1,7) = .026$; $P = .876$
	7	$F(1,7) = 16.142$; $P = .005$ **	$F(1,7) = 13.621$; $P = .008$ [#]	$F(1,7) = .060$; $P = .814$
	8	$F(1,7) = .645$; $P = .448$	$F(1,7) = 4.914$; $P = .062$	$F(1,7) = .000$; $P = .995$
9 mg/kg	1	$F(1,7) = 2.573$; $P = .153$	$F(1,7) = 1.750$; $P = .227$	$F(1,7) = .232$; $P = .645$
	3	$F(1,7) = 20.541$; $P = .003$ **	$F(1,7) = 14.506$; $P = .007$ [#]	$F(1,7) = .000$; $P = .983$
	5	$F(1,7) = 11.616$; $P = .011$ *	$F(1,7) = 1.938$; $P = .207$	$F(1,7) = .066$; $P = .805$
	7	$F(1,7) = 23.600$; $P = .002$ **	$F(1,7) = 1.105$; $P = .328$	$F(1,7) = .051$; $P = .828$
	8	$F(1,7) = 43.553$; $P = .000$ ***	$F(1,7) = 19.335$; $P = .003$ [#]	$F(1,7) = .031$; $P = .865$

Treatment: DGAT-1i (3 and 9 mg/kg) vs. vehicle; Diet: HFD vs. chow;
* Significant *treatment* effect (* $P < .05$, ** $P < .01$, *** $P < .001$), [#] Significant *treatment x diet* interaction ([#] $P < .01$)

Supplementary Table 2. Mann-Whitney Test
Grouping variable Diet (HFD vs. Chow)

Metabolite	Treatment	After IG infusion (h)	Chow		HFD		*U*	*P* - Value
			MR	Mdn	MR	Mdn		
TG	Vehicle	Baseline	5.71	0.197	9.29	0.260	12.0	0.128
		2	4.00	0.93	11.00	2.131	0.0	0.001*
		3	4.86	1.477	10.14	2.879	6.0	0.017*
		5	6.00	0.754	9.00	0.865	14.0	0.209
	DGAT-1i	Baseline	6.43	0.260	8.57	0.218	17.0	0.383
		2	8.43	0.873	6.57	0.690	18.0	0.456
		3	10.71	1.145	4.29	0.625	2.0	0.002*
		5	9.00	0.917	6.00	0.637	14.0	0.209
Free Glycerol	Vehicle	Baseline	6.29	0.702	8.71	0.764	16.0	0.318
		2	4.29	0.066	10.71	0.183	2.0	0.002*
		3	4.29	0.073	10.71	0.200	2.0	0.002*
		5	4.86	0.076	10.14	0.123	6.0	0.017*
	DGAT-1i	Baseline	9.0	0.772	6.0	0.579	14.0	0.197
		2	5.43	0.044	9.57	0.082	10.0	0.073
		3	4.43	0.065	10.57	0.171	3.0	0.004*
		5	4.0	0.089	11.00	0.159	0.0	0.001*
NEFA	Vehicle	Baseline	6.29	2.151	8.71	2.293	16.0	0.318
		2	4.43	0.087	10.57	0.494	3.0	0.004*
		3	4.0	0.092	11.0	0.474	0.0	0.001*
		5	5.71	0.297	9.29	0.335	12.0	0.128
	DGAT-1i	Baseline	9.0	2.376	6.0	1.682	14.0	0.209
		2	4.71	0.084	10.29	0.386	5.0	0.011*
		3	4.0	0.101	11.0	0.628	0.0	0.001*
		5	4.0	0.225	11.0	0.585	0.0	0.001*
BHB	Vehicle	Baseline	8.71	124.0	6.29	80.0	16.0	0.318
		2	4.0	38.0	11.0	178.0	0.0	0.001*
		3	4.0	39.0	11.0	181.0	0.0	0.001*
		5	5.29	73.0	9.71	161.0	9.0	0.53
	DGAT-1i	Baseline	4.71	125.0	10.29	336.0	5.0	0.011*
		2	4.0	45.0	11.0	339.0	0.0	0.001*
		3	4.0	39.0	11.0	445.0	0.0	0.001*
		5	4.0	74.0	11.0	574.0	0.0	0.001*

MR, Mean Rank; Mdn, Median
* *P* < .05, Exact Sig. (2-tailed)

Supplementary Table 3. Wilcoxon signed ranks test
Testing variable Time (2, 3, 5 h after infusion vs. Baseline)

Metabolite	Diet	Treatment	After IG infusion (h) 2 Mdn	3 Mdn	5 Mdn	Baseline Mdn	Z	P - Value
TG	Chow	Vehicle	0.930			0.197	- 2.366[a]	0.016*
				1.477		0.197	- 2.366[a]	0.016*
					0.754	0.197	- 2.366[a]	0.016*
		DGAT-1i	0.873			0.260	- 2.366[a]	0.016*
				1.145		0.260	- 2.366[a]	0.016*
					0.917	0.260	- 2.371[a]	0.016*
	HFD	Vehicle	2.131			0.260	- 2.366[a]	0.016*
				2.879		0.260	- 2.366[a]	0.016*
					0.865	0.260	- 2.366[a]	0.016*
		DGAT-1i	0.690			0.218	- 2.366[a]	0.016*
				0.625		0.218	- 2.366[a]	0.016*
					0.637	0.218	- 2.366[a]	0.016*
Free Glycerol	Chow	Vehicle	0.066			0.702	- 2.366[b]	0.016*
				0.073		0.702	- 2.366[b]	0.016*
					0.076	0.702	- 2.366[b]	0.016*
		DGAT-1i	0.044			0.772	- 2.366[b]	0.016*
				0.065		0.772	- 2.366[b]	0.016*
					0.065	0.772	- 2.366[b]	0.016*
	HFD	Vehicle	0.183			0.764	- 2.366[b]	0.016*
				0.200		0.764	- 2.366[b]	0.016*
					0.123	0.764	- 2.366[b]	0.016*
		DGAT-1i	0.579			0.579	- 2.366[b]	0.016*
				0.082		0.579	- 2.371[b]	0.016*
					0.171	0.579	- 2.366[b]	0.016*
NEFA	Chow	Vehicle	0.087			2.151	- 2.371[b]	0.016*
				0.092		2.151	- 2.366[b]	0.016*
					0.297	2.151	- 2.366[b]	0.016*
		DGAT-1i	0.084			2.376	- 2.366[b]	0.016*
				0.101		2.376	- 2.366[b]	0.016*
					0.225	2.376	- 2.366[b]	0.016*
	HFD	Vehicle	0.494			2.293	- 2.366[b]	0.016*
				0.474		2.293	- 2.366[b]	0.016*
					0.335	2.293	- 2.366[b]	0.016*
		DGAT-1i	0.386			1.682	- 2.366[b]	0.016*
				0.628		1.682	- 2.366[b]	0.016*
					0.585	1.682	- 2.366[b]	0.016*
BHB	Chow	Vehicle	38.00			124.0	- 2.371[b]	0.016*
				39.00		124.0	- 2.371[b]	0.016*
					73.00	124.0	- 2.197[b]	0.031*
		DGAT-1i	45.00			125.0	- 2.197[b]	0.031*
				39.00		125.0	- 2.201[b]	0.031*
					74.00	125.0	- 1.863[b]	0.078
	HFD	Vehicle	178.0			80.00	- 0.676[a]	0.578
				181.0		80.00	- 1.183[a]	0.297
					161.0	80.00	- 0.507[a]	0.688
		DGAT-1i	339.0			336.0	- 1.153[a]	0.313
				445.0		336.0	- 2.366[a]	0.016*
					574.0	336.0	- 1.859[a]	0.078

Mdn, Median
a. Based on negative ranks (2, 3 & 5 h < Baseline)
b. Based on positive ranks (2, 3 & 5 h > Baseline)
* P < .05, Exact Sig. (2-tailed)

Supplementary Table 4. Wilcoxon signed ranks test
Testing variable Treatment (DGAT-1i vs. Vehicle)

Metabolite	Diet	After IG infusion (h)	DGAT-1i Mdn	Vehicle Mdn	Z	P - Value
TG	Chow	Baseline	0.260	0.197	- 0.338[a]	0.813
		2	0.873	0.93	- 1.270[a]	0.234
		3	1.145	1.477	- 0.845[a]	0.469
		5	0.917	0.754	- 2.028[b]	0.047*
	HFD	Baseline	0.218	0.260	- 0.169[a]	0.938
		2	0.690	2.131	- 2.366[a]	0.016*
		3	0.625	2.879	- 2.336[a]	0.016*
		5	0.637	0.865	- 1.183[a]	0.297
Free Glycerol	Chow	Baseline	0.772	0.702	- 0.845[b]	0.469
		2	0.044	0.066	- 0.423[a]	0.734
		3	0.065	0.073	- 0.507[a]	0.688
		5	0.089	0.076	- 0.507[b]	0.688
	HFD	Baseline	0.579	0.764	- 1.352[a]	0.219
		2	0.082	0.183	- 2.028[a]	0.047*
		3	0.171	0.200	- 1.521[a]	0.156
		5	0.159	0.123	-2.028[b]	0.047*
NEFA	Chow	Baseline	2.376	2.151	- 1.014[b]	0.375
		2	0.084	0.087	- 0.676[a]	0.578
		3	0.101	0.092	- 1.272[a]	0.250
		5	0.225	0.297	- 1.609[a]	0.125
	HFD	Baseline	1.682	2.293	- 1.521[a]	0.156
		2	0.386	0.494	- 1.183[a]	0.297
		3	0.628	0.474	- 1.439[b]	0.172
		5	0.585	0.335	- 2.366[b]	0.016*
BHB	Chow	Baseline	125.0	124.0	- 0.507[b]	0.688
		2	45.0	38.0	- 1.442[b]	0.188
		3	39.0	39.0	- 0.508[b]	0.672
		5	74.0	73.0	- 0.676[b]	0.578
	HFD	Baseline	336.0	80.0	- 2.366[b]	0.016*
		2	339.0	178.0	- 2.366[b]	0.016*
		3	445.0	181.0	- 2.366[b]	0.016*
		5	574.0	161.0	- 2.366[b]	0.016*

Mdn, Median
a. Based on positive ranks (DGAT-1i > Vehicle)
b. Based on negative ranks (DGAT-1i < Vehicle)
* P < .05, Exact Sig. (2-tailed)

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Supplementary Table 5A. Mann-Whitney Test
Grouping variable Treatment (DGAT-1i vs. Vehicle)

Tissue	Protein	Diet	Vehicle		DGAT-1i		U	P - Value
			MR	Mdn	MR	Mdn		
Liver	CPT-1	Chow	5.50	102.9774	3.50	89.6743	4.0	0.343
		HFD	4.75	190.2010	4.25	177.5755	7.0	0.886
	p-AMPK/AMPK ratio	Chow	4.75	94.4225	4.25	91.0241	7.0	0.886
		HFD	5.00	318.3117	4.00	296.5340	6.0	0.686
	HMG-CoAS2	Chow	5.50	96.3569	3.50	83.3159	4.0	0.343
		HFD	5.75	107.0562	3.25	72.2746	3.0	0.200
	Complex III	Chow	6.00	95.6690	3.00	75.6652	2.0	0.114
		HFD	4.50	100.2408	4.50	100.3510	8.0	1.000
Duodenum	HMG-CoAS2	Chow	5.00	99.1405	4.00	100.2976	6.0	0.686
		HFD	3.75	143.8945	5.25	160.2699	5.0	0.486
	Complex III	Chow	4.00	112.2900	5.00	259.0919	6.0	0.686
		HFD	3.00	1586.8068	6.00	4317.5881	2.0	0.114
Jejunum	HMG-CoAS2	Chow	4.75	105.6665	4.25	100.6998	7.0	0.886
		HFD	2.50	177.3431	6.50	288.7063	0.0	0.029*
	Complex III	Chow	3.50	76.6257	5.50	124.0154	4.0	0.343
		HFD	2.50	135.7654	6.50	475.9557	0.0	0.029*

Supplementary Table 5B. Mann-Whitney Test
Grouping variable Diet (HFD vs. Chow)

Tissue	Protein	Treatment	Chow		HFD		U	P - Value
			MR	Mdn	MR	Mdn		
Liver	CPT-1	Vehicle	2.50	102.9774	6.50	190.3453	0.0	0.029[#]
		DGAT-1i	2.50	89.6734	6.50	177.5755	0.0	0.029[#]
	p-AMPK/AMPK ratio	Vehicle	2.50	94.4225	6.50	318.3117	0.0	0.029[#]
		DGAT-1i	2.50	91.0241	6.50	296.5340	0.0	0.029[#]
	HMG-CoAS2	Vehicle	4.0	96.3569	5.0	107.0562	6.0	0.686
		DGAT-1i	5.75	83.3159	3.25	72.2746	3.0	0.200
	Complex III	Vehicle	4.25	95.6690	4.75	100.2408	7.0	0.886
		DGAT-1i	4.00	75.6652	5.00	100.3510	6.0	0.686
Duodenum	HMG-CoAS2	Vehicle	2.50	99.1405	6.50	143.8945	0.0	0.029[#]
		DGAT-1i	2.50	100.2976	6.50	160.2699	0.0	0.029[#]
	Complex III	Vehicle	2.50	112.2900	6.50	1586.8068	0.0	0.029[#]
		DGAT-1i	2.50	259.0919	6.50	4317.5881	0.0	0.029[#]
Jejunum	HMG-CoAS2	Vehicle	2.50	105.6665	6.50	177.3431	0.0	0.029[#]
		DGAT-1i	2.50	100.6998	6.50	288.7063	0.0	0.029[#]
	Complex III	Vehicle	3.75	76.6257	5.25	135.7654	5.0	0.486
		DGAT-1i	2.50	124.0154	6.50	475.9557	0.0	0.029[#]

MR, Mean Rank; Mdn, Median
* P < .05 (Vehicle vs. DGAT-1i within diet group); [#] P < .05 (Chow vs. HFD within treatment group); Exact Sig. (2-tailed)

Supplementary Table 6. Best-fit parameter values of non-linear regression

		DGAT-1i [μM]	Best-fit values		Std. Error	95% CI		Goodness of Fit	
			$LogEC_{50}$	EC_{50}	$LogEC_{50}$	$LogEC_{50}$	EC_{50}	df	R^2
CaCo2 [a]	^{14}C-Palmitate	0.001-10	-0.457	0.3494	0.0947	-0.653 to -0.261	0.223 to 0.549	23	0.725
HuTu80 [b]		0.001-10	-2.111	0.0078*	0.2768	-2.680 to -1.542	0.002 to 0.029	26	0.638
HuTu80 [b]	^{14}C-Oleate	0.0001-0.1	-2.197	0.0064*	0.0363	-2.272 to -2.122	0.005 to 0.008	24	0.991
HuTu80 [b]		0.0001-1	-2.059	0.0087*	0.0419	-2.145 to -1.972	0.007 to 0.011	24	0.987

Best-fit values for concentration-response curves of CaCo2 and HuTu80 cell lines incubated with different DGAT-1i concentrations. [a] log(agonist) vs. response equation for four parameters, [b] log(agonist) vs. response equation for three-parameters; * Significant difference between cell lines; Bonferroni post-hoc test ($P < .001$ vs. CaCo2 ^{14}C-Palmitate) after significant one-way ANOVA ($F(3,97) = 27.22$, $P < .001$).
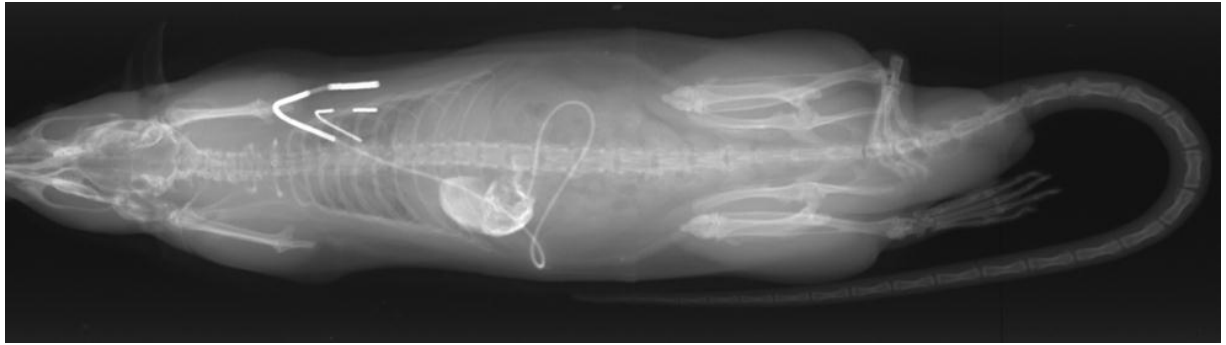
Supplementary Fig. 1. Intragastric (IG) catheter verification by x-ray scans (LaTheta LCT-100 CT scanner). Rats were fasted overnight and x-ray scans were taken after IG infusion of diluted contrast agent (1:2; Accupaque TM, GE healthcare, UK; 0.6 ml, 2 parts Accupaque + 1 part saline) under isoflurane inhalation anesthesia. The representative picture shows the accumulation of the contrast agent in the stomach indicating an intact IG catheter.
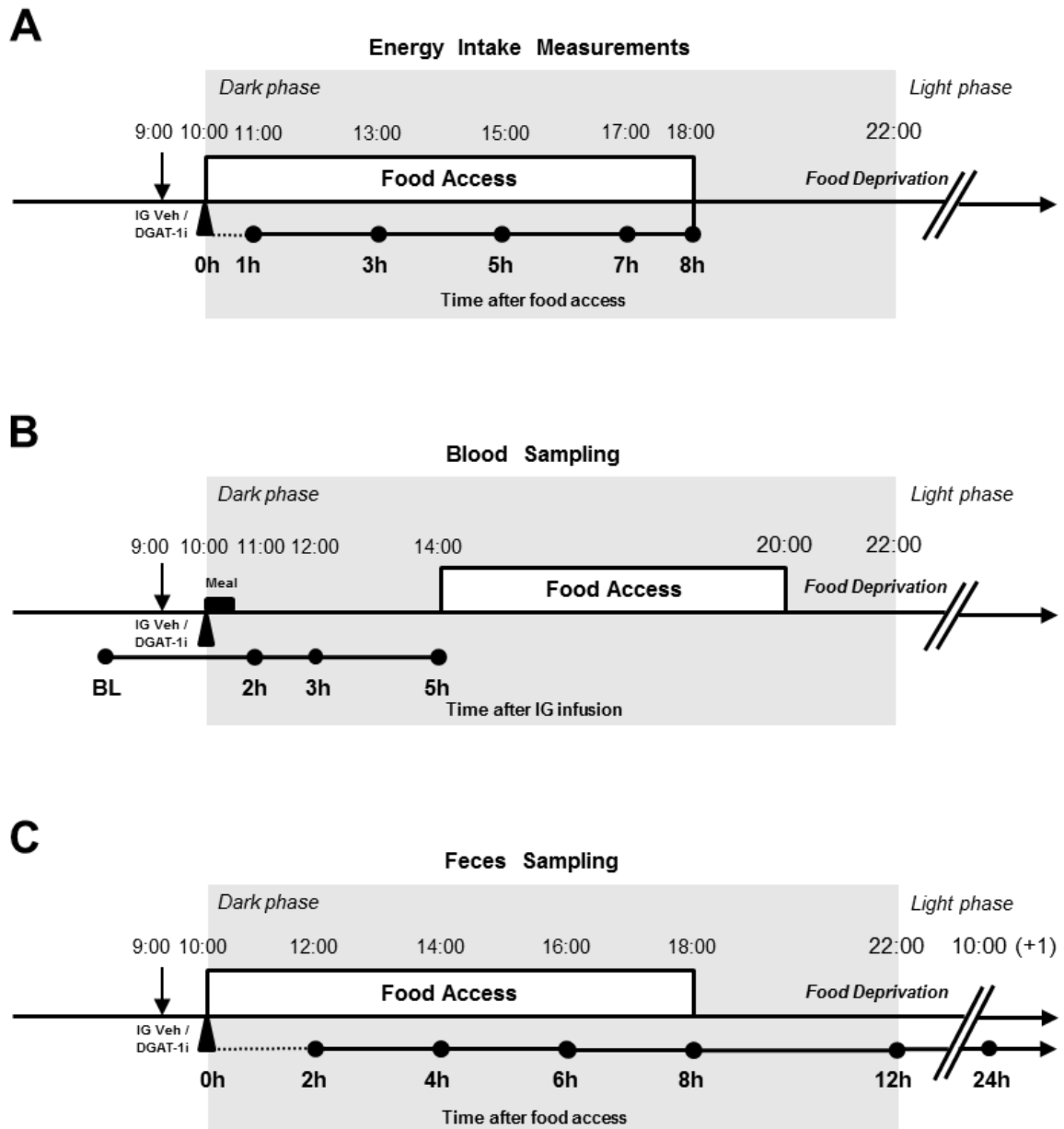
Supplementary Fig. 2. Experimental design of (A) energy intake, (B) blood sampling and (C) feces sampling experiments. Acute intragastric (IG) vehicle (Veh) or DGAT-1i infusions (↓; 5 ml/kg) were always performed 1 hour prior dark onset. At dark onset (▲, 10:00) either food access was established for 8 h (A and C) or rats received an iso-caloric test meal (■; B), consisting of 3 g HFD or 5 g chow after 16 h food deprivation. Energy intake measurements or blood and feces samplings were performed at the times indicated by solid circles (●). Shaded areas indicate dark phase. BL, base line; HFD, high fat diet
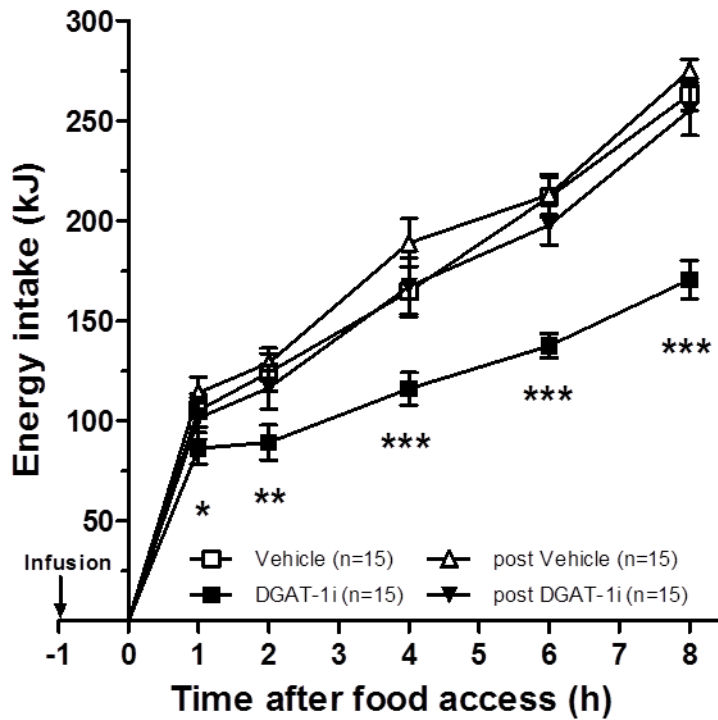
Supplementary Fig. 3. The eating inhibitory effect of IG DGAT-1i on HFD-fed rats was not compensated on the next day when rats were left untreated. The energy intake was measured every 1 – 2 hours after IG DGAT-1i (10 mg/kg; ■) or vehicle (□) infusions (↓; 5 ml/kg) during the 8 h feeding period. Energy intake measurements were also performed on the subsequent day when no IG infusions took place (post vehicle, Δ; post DGAT-1i, ▼). Data are shown as means ± SEM and were analyzed for each day separately by paired t-test. Comparisons were made for individual time points. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. vehicle.
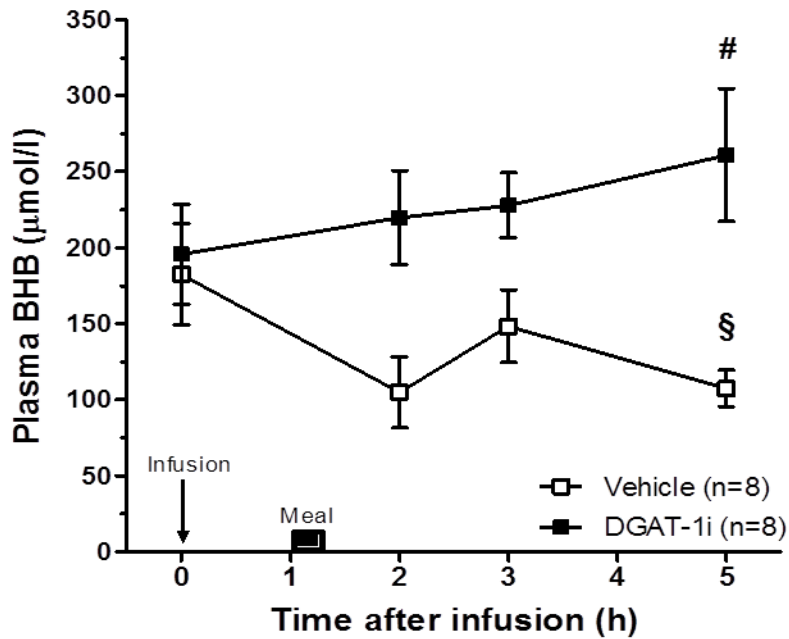
Supplementary Fig. 4. Acute IG DGAT-1i infusions increased circulating plasma BHB levels in HFD-fed rats, which is indicative for induced ketogenesis. Rats were food deprived for 16 h during light phase and re-fed with a 5 g HFD meal at dark onset (10:00). Acute IG infusions ($\downarrow$; 5 ml/kg) of DGAT-1i (9 mg/kg; ■) or vehicle (□) were given 1 h before dark onset. Data are means ± SEM for 8 animals per group. Analysis of plasma BHB across all four time points by a 2 × 4 (treatment × time) repeated measures ANOVA, yielded a significant treatment effect ($P < 0.05$) and *treatment × diet* interaction ($P < 0.05$). [#] $P < 0.05$ vs. vehicle, [§] $P < 0.05$ vs. baseline (time 0) in Bonferroni Holm post-hoc tests after significant ANOVA.