Electronic supplementary material

Clinical Pharmacokinetics

Safety and pharmacokinetics of single and multiple ascending doses of the novel oral human GLP-1 analogue, oral semaglutide, in healthy subjects and subjects with type 2 diabetes

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Table S1 Pharmacokinetic blood sampling scheme (single-dose trial)

Nominal time	Semaglutide	SNAC
00:00[a]	X	X
00:15	X	X
00:30	X	X
01:00	X	X
01:30	X	X
02:00	X	X
02:30	X	X
03:00	X	X
03:30	X	X
04:00	X	X
04:30	X	X
05:00	X	X
05:30	X	
06:00	X	
07:00	X	
08:00	X	
10:00	X	
12:00	X	
16:00	X	
24:00	X	
36:00	X	
48:00	X	
60:00	X	
72:00	X	
84:00	X	
96:00	X	
108:00	X	
120:00	X	
144:00	X	
168:00	X	
264:00	X	
360:00	X	
504:00	X	

[a] Pre-dose.
SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate.

Table S2 Pharmacokinetic blood sampling scheme (multiple-dose trial)

Day	Nominal time[a]	Semaglutide	SNAC	Day	Nominal time[a]	Semaglutide	SNAC
0	00:00[b]	X	X	68	00:00[b]	X	X
	00:10		X		00:10		X
	00:20		X		00:20		X
	00:30		X		00:30	X	X
	00:40		X		00:40		X
	00:50		X		00:50		X
	01:00		X		01:00	X	X
	01:30		X		01:30		X
	02:00		X		02:00	X	X
	02:30		X		02:30		X
	03:00		X		03:00	X	X
	04:00		X		06:00	X	X
	06:00		X		12:00	X	
7	00:00[b]	X[c]	X	69	00:00[b]	X	X
14	00:00[b]	X[c]	X		00:10		X
21	00:00[b]	X[c]	X		00:20		X
28	00:00[b]	X[c]	X		00:30	X	X
35	00:00[b]	X[c]	X		00:40		X
42	00:00[b]	X[c]	X		00:50		X
49	00:00[b]	X[c]	X		01:00	X	X
56	00:00[b]	X[c]	X		01:30		X
63	00:00[b]	X[c]	X		02:00	X	X
67	00:00[b]	X	X		02:30		X
	00:10		X		03:00	X	X
	00:20		X		06:00	X	X
	00:30	X	X		12:00	X	
	00:40		X		24:00	X	X
	00:50		X		48:00	X	X
	01:00	X	X		96:00	X	X
	01:30		X		168:00	X	X
	02:00	X	X		240:00	X	X
	02:30		X		336:00	X	X
	03:00	X	X		504:00[d]	X	X
	06:00	X	X				
	12:00	X	X				

[a] Relative to dosing on the respective day; [b] Pre-dose; [c] An additional sample was taken at 1 hour post-dose; [d] This sample was taken between 504 and 840 hours after the last dose.
SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate.

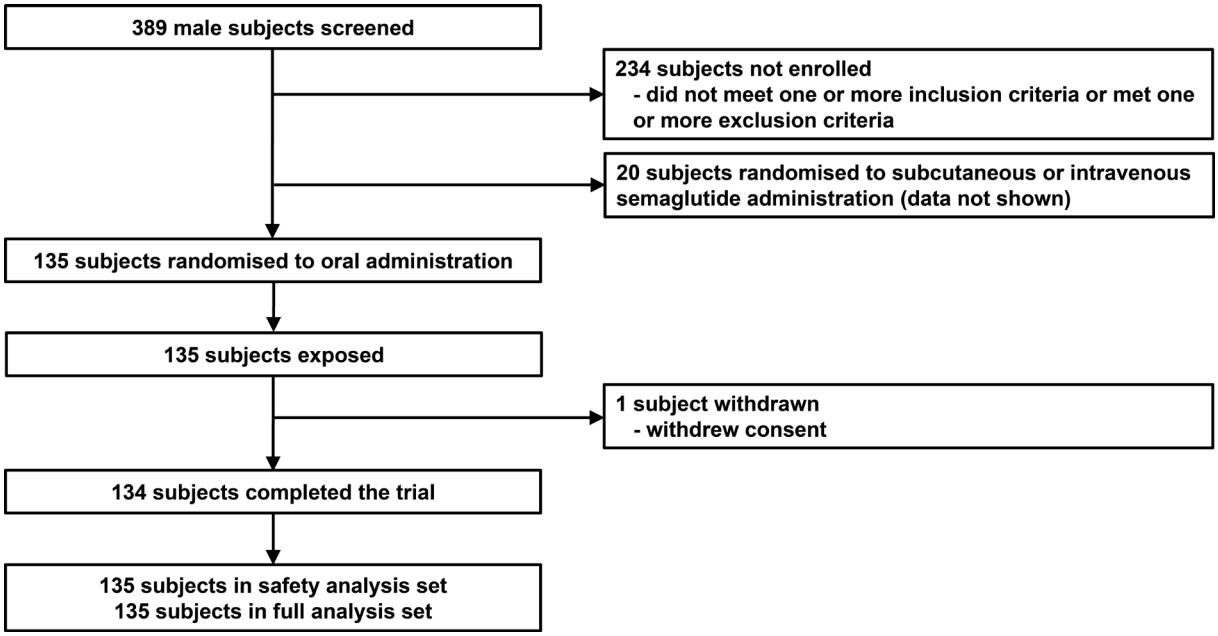

Fig. S1 Subject disposition for the single-dose trial

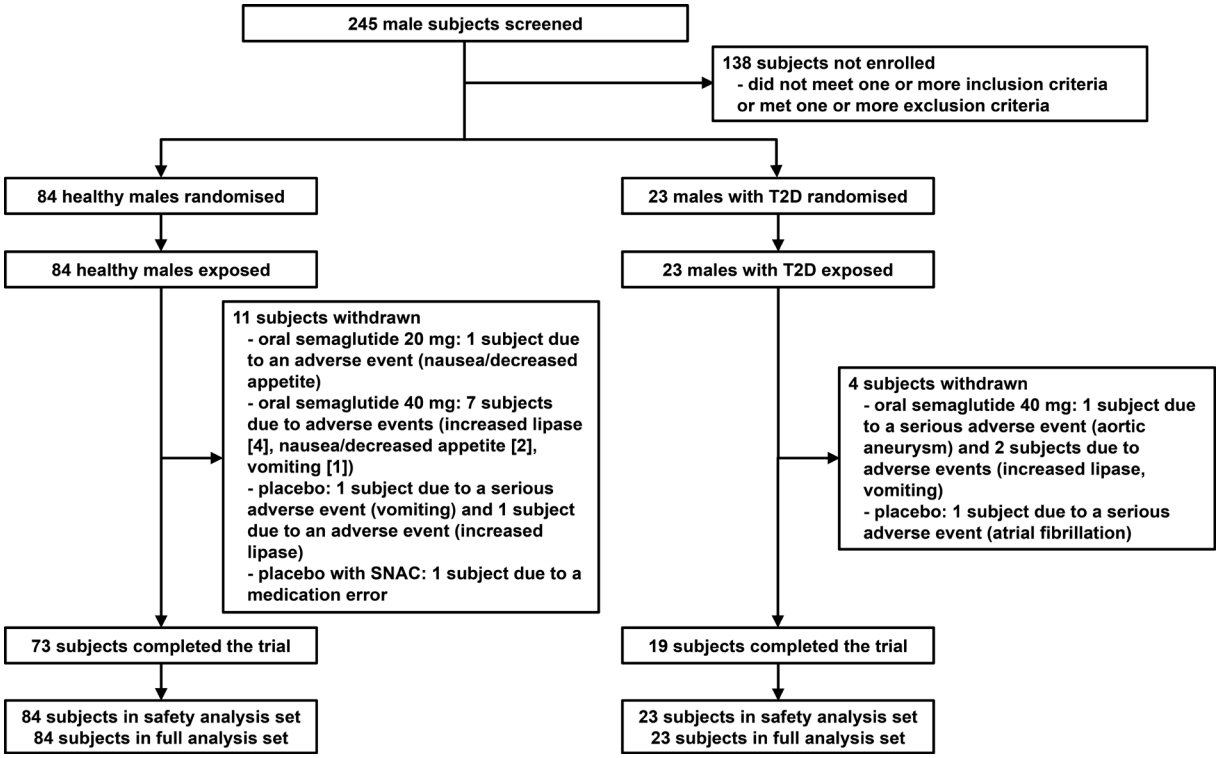

Fig. S2 Subject disposition for the multiple-dose trial

T2D, type 2 diabetes.

Table S3 Subject characteristics for each treatment arm in the single-dose trial

	2 mg oral semaglutide/ 300 mg SNAC	5 mg oral semaglutide/ 150 mg SNAC	5 mg oral semaglutide/ 300 mg SNAC	10 mg oral semaglutide/ 300 mg SNAC	10 mg oral semaglutide/ 600 mg SNAC	15 mg oral semaglutide/ 450 mg SNAC	20 mg oral semaglutide/ 600 mg SNAC	Placebo with SNAC[a]
	N=24	N=10	N=24	N=24	N=10	N=10	N=10	N=23
Age, years	31.4 (8.6)	31.4 (4.4)	29.7 (9.0)	30.0 (8.6)	30.1 (8.8)	26.1 (4.7)	34.8 (8.2)	28.3 (6.8)
Sex								
Male, N (%)	24 (100.0)	10 (100.0)	24 (100.0)	24 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	23 (100.0)
Race								
White, N (%)	13 (54.2)	8 (80.0)	19 (79.2)	16 (66.7)	7 (70.0)	9 (90.0)	5 (50.0)	16 (69.6)
Asian, N (%)	4 (16.7)	1 (10.0)	2 (8.3)	4 (16.7)	0 (0.0)	0 (0.0)	2 (20.0)	3 (13.0)
Black or African American, N (%)	3 (12.5)	0 (0.0)	2 (8.3)	0 (0.0)	1 (10.0)	0 (0.0)	2 (20.0)	3 (13.0)
Other, N (%)	4 (16.7)	1 (10.0)	1 (4.2)	4 (16.7)	2 (20.0)	1 (10.0)	1 (10.0)	1 (4.3)
Body weight, kg	74.8 (6.5)	76.2 (7.2)	75.8 (8.2)	74.4 (6.6)	70.7 (3.7)	74.4 (6.7)	72.5 (9.8)	73.5 (6.6)
BMI, kg/m^2	24.2 (1.8)	24.4 (1.3)	23.6 (2.1)	23.2 (2.3)	23.1 (2.2)	24.0 (1.9)	24.5 (2.0)	23.6 (2.4)

[a] The different amounts of SNAC have been pooled.
Data are mean (standard deviation) unless otherwise stated.
BMI, body mass index; N, number of subjects; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate.

Table S4 Subject characteristics for each treatment arm in the multiple-dose trial

	Healthy males				Males with T2D		
	20 mg oral semaglutide	40 mg oral semaglutide	Placebo[a]	Placebo with SNAC[b]	40 mg oral semaglutide	Placebo	Placebo with SNAC
	N=16	N=32	N=18	N=18	N=11	N=6	N=6
Age, years	49.4 (9.5)	40.8 (10.7)	46.4 (14.5)	45.7 (10.5)	55.3 (8.1)	55.5 (11.3)	52.2 (6.1)
Sex							
Male, N (%)	16 (100.0)	32 (100.0)	18 (100.0)	18 (100.0)	11 (100.0)	6 (100.0)	6 (100.0)
Race							
White, N (%)	16 (100.0)	30 (93.8)	18 (100.0)	18 (100.0)	10 (90.9)	6 (100.0)	6 (100.0)
Black or African American, N (%)	0 (0.0)	1 (3.1)	0 (0.0)	0 (0.0)	1 (9.1)	0 (0.0)	0 (0.0)
Other, N (%)	0 (0.0)	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Body weight, kg	84.2 (12.2)	83.9 (9.4)	83.6 (8.6)	81.9 (11.9)	92.4 (11.0)	97.9 (17.3)	96.7 (16.7)
BMI, kg/m^2	26.1 (2.7)	25.5 (2.5)	26.5 (1.9)	25.8 (2.6)	29.8 (2.4)	29.4 (4.2)	28.7 (3.9)
Duration of diabetes, years	NA	NA	NA	NA	6.2 (3.6)	6.9 (2.4)	3.4 (2.5)
HbA_{1c}, %	NA	NA	NA	NA	7.5 (0.7)	7.6 (0.9)	7.4 (0.6)

[a] The two placebo groups for healthy subjects have been pooled; [b] The two placebo with SNAC groups for healthy subjects have been pooled.
Data are mean (standard deviation) unless otherwise stated.
BMI, body mass index; HbA_{1c}, glycosylated haemoglobin; N, number of subjects; NA, not applicable; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate; T2D, type 2 diabetes.

Table S5 Summary of treatment-emergent adverse events after a single dose of oral semaglutide or placebo with SNAC in healthy male subjects (single-dose trial)

	2 mg oral semaglutide/ 300 mg SNAC			5 mg oral semaglutide/ 150 mg SNAC			5 mg oral semaglutide/ 300 mg SNAC			10 mg oral semaglutide/ 300 mg SNAC			10 mg oral semaglutide/ 600 mg SNAC			15 mg oral semaglutide/ 450 mg SNAC			20 mg oral semaglutide/ 600 mg SNAC			Placebo with SNAC[a]		
	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E
Total number of subjects	24			10			24			24			10			10			10			23		
Adverse events	10	(41.7)	21	5	(50.0)	6	10	(41.7)	16	12	(50.0)	28	5	(50.0)	9	2	(20.0)	3	5	(50.0)	10	6	(26.1)	11
Serious adverse events	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0
Severity																								
Severe	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0
Moderate	1	(4.2)	1	0	(0.0)	0	2	(8.3)	2	2	(8.3)	2	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0
Mild	10	(41.7)	20	5	(50.0)	6	8	(33.3)	14	12	(50.0)	26	5	(50.0)	9	2	(20.0)	3	5	(50.0)	10	6	(26.1)	11
Relationship to trial product																								
Probable	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0
Possible	3	(12.5)	9	2	(20.0)	2	5	(20.8)	8	9	(37.5)	17	4	(40.0)	5	1	(10.0)	1	3	(30.0)	3	4	(17.4)	6
Unlikely	10	(41.7)	12	4	(40.0)	4	5	(20.8)	8	9	(37.5)	11	2	(20.0)	4	1	(10.0)	2	4	(40.0)	7	3	(13.0)	5
Outcome																								
Not recovered	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0
Recovering	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0
Recovered	10	(41.7)	21	5	(50.0)	6	10	(41.7)	16	12	(50.0)	28	5	(50.0)	9	2	(20.0)	3	5	(50.0)	10	6	(26.1)	11

[a] The different amounts of SNAC have been pooled.
E, number of adverse events; N, number of subjects with at least one adverse event; %, percentage of subjects with at least one adverse event; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate.

Table S6 Summary of $AUC_{0\text{-}5h,SNAC,SD}$, $C_{max,SNAC,SD}$ and $t_{max,SNAC,SD}$ after a single dose of oral semaglutide in healthy male subjects (single-dose trial)

	5 mg oral semaglutide/ 150 mg SNAC	2 mg oral semaglutide/ 300 mg SNAC	5 mg oral semaglutide/ 300 mg SNAC	10 mg oral semaglutide/ 300 mg SNAC	15 mg oral semaglutide/ 450 mg SNAC	10 mg oral semaglutide/ 600 mg SNAC	20 mg oral semaglutide/ 600 mg SNAC
	N=10	N=24	N=24	N=24	N=10	N=10	N=10
$AUC_{0\text{-}5h,SNAC,SD}$ (ng·h/mL)							
Geometric mean	383.5	968.3	794.5	743.0	1360.6	1520.0	1821.0
CV%	30.8	26.2	34.5	30.7	25.1	51.1	28.4
Min-Max	162.2-604.8	599.2-1660.6	296.0-1537.1	300.8-1235.2	700.2-2029.8	761.7-3440.4	756.5-2567.6
$C_{max,SNAC,SD}$ (ng/mL)							
Geometric mean	432.2	870.9	760.9	724.1	938.7	1323.4	1673.2
CV%	61.5	46.9	67.0	49.8	51.9	88.0	49.7
Min-Max	105-1190	276-1988	197-3012	211-1729	505-1947	441-5367	469-3488
$t_{max,SNAC,SD}$ (hours)							
Median	0.38	0.50	0.50	0.38	0.75	0.50	0.50
Min - Max	0.25-3.00	0.25-5.00	0.25-3.50	0.25-5.00	0.25-2.50	0.25-1.00	0.25-1.00

AUC, area under the curve; C_{max}, maximum concentration; CV%, coefficient of variation in %; Max, maximum; Min, minimum; SD, single dose; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate; t_{max}, time to maximum concentration.

Table S7 Summary of the most frequently reported treatment-emergent adverse events per system organ class and preferred term during 10 weeks of once-daily dosing of oral semaglutide, placebo or placebo with SNAC in healthy males and in males with T2D (multiple-dose trial)

	Healthy males									Males with T2D								
System organ class	20 mg oral semaglutide			40 mg oral semaglutide			Placebo[a]			Placebo with SNAC[b]			40 mg oral semaglutide			Placebo			Placebo with SNAC		
Preferred term	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E	N	(%)	E
Total number of subjects	16			32			18			18			11			6			6		
Gastrointestinal disorders	8	(50.0)	70	27	(84.4)	148	5	(27.8)	14	13	(72.2)	46	8	(72.7)	42	3	(50.0)	5	2	(33.3)	3
Nausea	7	(43.8)	8	15	(46.9)	25	3	(16.7)	4	4	(22.2)	7	6	(54.5)	7	0	(0.0)	0	0	(0.0)	0
Vomiting	5	(31.3)	17	13	(40.6)	20	2	(11.1)	2	1	(5.6)	1	5	(45.5)	11	0	(0.0)	0	0	(0.0)	0
Eructation	2	(12.5)	7	14	(43.8)	27	0	(0.0)	0	1	(5.6)	2	3	(27.3)	4	0	(0.0)	0	0	(0.0)	0
Abdominal pain	3	(18.8)	9	9	(28.1)	14	2	(11.1)	3	5	(27.8)	12	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0
Diarrhoea	5	(31.3)	11	4	(12.5)	8	1	(5.6)	1	3	(16.7)	4	2	(18.2)	2	1	(16.7)	2	2	(33.3)	3
Abdominal distention	3	(18.8)	3	7	(21.9)	9	1	(5.6)	2	4	(22.2)	6	1	(9.1)	1	0	(0.0)	0	0	(0.0)	0
Dyspepsia	1	(6.3)	2	10	(31.3)	13	0	(0.0)	0	1	(5.6)	2	4	(36.4)	8	0	(0.0)	0	0	(0.0)	0
Abdominal discomfort	2	(12.5)	4	5	(15.6)	5	0	(0.0)	0	2	(11.1)	2	3	(27.3)	3	0	(0.0)	0	0	(0.0)	0
Flatulence	1	(6.3)	7	4	(12.5)	6	0	(0.0)	0	3	(16.7)	6	1	(9.1)	1	1	(16.7)	2	0	(0.0)	0
Abdominal pain upper	1	(6.3)	1	3	(9.4)	6	1	(5.6)	1	0	(0.0)	0	1	(9.1)	1	0	(0.0)	0	0	(0.0)	0
Infrequent bowel movements	0	(0.0)	0	3	(9.4)	5	1	(5.6)	1	2	(11.1)	2	0	(0.0)	0	0	(0.0)	0	0	(0.0)	0
Toothache	0	(0.0)	0	4	(12.5)	4	0	(0.0)	0	1	(5.6)	1	0	(0.0)	0	1	(16.7)	1	0	(0.0)	0
Metabolism and nutrition disorders	10	(62.5)	12	25	(78.1)	33	2	(11.1)	3	4	(22.2)	7	10	(90.9)	10	0	(0.0)	0	2	(33.3)	2
Decreased appetite	10	(62.5)	12	25	(78.1)	33	2	(11.1)	3	3	(16.7)	6	10	(90.9)	10	0	(0.0)	0	2	(33.3)	2
Infections and infestations	2	(12.5)	2	12	(37.5)	16	9	(50.0)	11	8	(44.4)	13	2	(18.2)	3	3	(50.0)	3	3	(50.0)	3
Nasopharyngitis	2	(12.5)	2	10	(31.3)	13	8	(44.4)	8	8	(44.4)	9	2	(18.2)	3	3	(50.0)	3	3	(50.0)	3
Nervous system disorders	5	(31.3)	18	14	(43.8)	34	5	(27.8)	8	6	(33.3)	17	5	(45.5)	8	1	(16.7)	1	1	(16.7)	1

Headache	5 (31.3)	16	12 (37.5)	24	5 (27.8)	8	6 (33.3)	17	4 (36.4)	7	0 (0.0)	0	1 (16.7)	1
General disorders and administration site conditions	6 (37.5)	10	10 (31.3)	18	2 (11.1)	2	5 (27.8)	9	3 (27.3)	4	1 (16.7)	1	2 (33.3)	2
Fatigue	4 (25.0)	5	8 (25.0)	10	1 (5.6)	1	3 (16.7)	7	2 (18.2)	3	1 (16.7)	1	1 (16.7)	1
Asthenia	3 (18.8)	4	3 (9.4)	4	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Investigations	0 (0.0)	0	7 (21.9)	8	1 (5.6)	1	0 (0.0)	0	1 (9.1)	1	1 (16.7)	1	0 (0.0)	0
Lipase increased	0 (0.0)	0	7 (21.9)	7	1 (5.6)	1	0 (0.0)	0	1 (9.1)	1	1 (16.7)	1	0 (0.0)	0

Only preferred terms with an overall frequency ≥5.0% are included.
[a] The two placebo groups for healthy subjects have been pooled.
[b] The two placebo with SNAC groups for healthy subjects have been pooled.
E, number of adverse events; N, number of subjects with at least one adverse event; %, percentage of subjects with at least one adverse event; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate; T2D, type 2 diabetes.

Table S8 Within-subject day-to-day variability and total variability in $AUC_{0\text{-}24h,semaglutide,SS}$ and $C_{max,semaglutide,SS}$ in healthy males and in males with T2D (multiple-dose trial)

	Healthy males		Males with T2D
	20 mg oral semaglutide	40 mg oral semaglutide	40 mg oral semaglutide
	N=16	N=32	N=11
$AUC_{0\text{-}24h,semaglutide,SS}$			
Within-subject day-to-day variability	19.7 [15.7;26.5]	32.6 [27.0;41.1]	25.3 [18.7;39.4]
Total variability	72.7 [51.9;126.4]	84.4 [64.5;124.6]	65.0 [43.4;138.4]
$C_{max,semaglutide,SS}$			
Within-subject day-to-day variability	24.1 [19.2;32.6]	34.9 [29.0;44.1]	31.0 [22.8;48.6]
Total variability	71.5 [51.6;121.0]	83.1 [64.0;121.1]	63.6 [43.5;125.4]

Data are estimated CV% and the 95% CI of the CV. Total variability consists of both the within-subject day-to-day variability and the between-subject variability.
AUC, area under the curve; CI, confidence interval; C_{max}, maximum concentration; CV%, coefficient of variation in %; N, number of subjects; %, percentage of subjects; SS, steady state; T2D, type 2 diabetes.

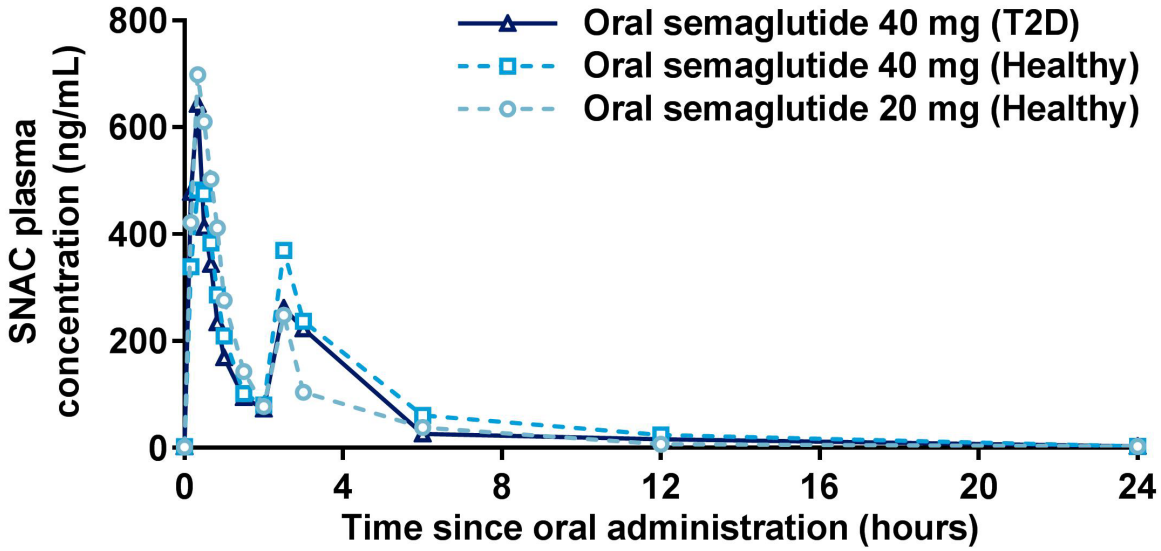

Fig. S3 Arithmetic mean SNAC plasma concentration-time profiles at steady state of oral semaglutide in healthy males and in males with T2D (multiple-dose trial)

Profiles represent arithmetic means of the last 3 days of once-daily oral semaglutide treatment for 10 weeks. T2D, type 2 diabetes.

Table S9 Summary of $AUC_{0\text{-}24h,SNAC,SS}$, $C_{max,SNAC,SS}$ and $t_{max,SNAC,SS}$ for oral semaglutide in healthy males and in males with T2D (multiple-dose trial)

	Healthy males 20 mg oral semaglutide N=16	Healthy males 40 mg oral semaglutide N=32	Males with T2D 20 mg oral semaglutide N=11
$AUC_{0\text{-}24h,SNAC,SS}$ (ng·h/mL)			
Geometric mean	1382.7	1636.3	1206.2
CV%	32.8	54.1	53.8
Min-Max	827.5-2861.5	938.9-5554.4	722.0-3528.5
$C_{max,SNAC,SS}$ (ng/mL)			
Geometric mean	657.9	606.6	578.8
CV%	86.1	79.0	191.3
Min-Max	161-1915	200-2236	76-4159
$t_{max,SNAC,SS}$ (hours)			
Median	0.50	0.50	1.43
Min-Max	0.2-2.5	0.2-6.0	0.2-3.0

Data are from the last day of once-daily oral semaglutide treatment for 10 weeks.
AUC, area under the curve; C_{max}, maximum concentration; CV%, coefficient of variation in %; Max, maximum; Min, minimum; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate; SS, steady state; T2D, type 2 diabetes; t_{max}, time to maximum concentration.