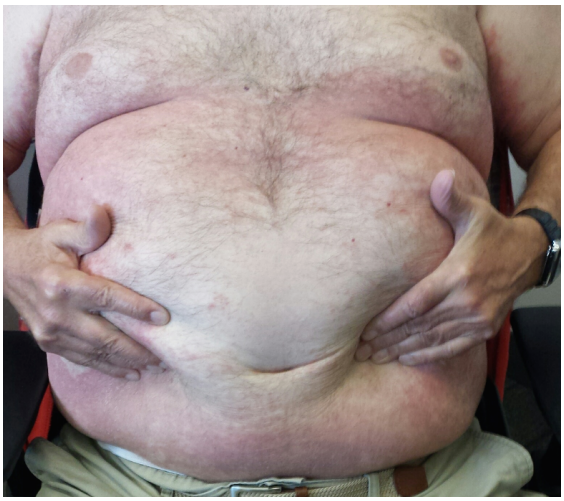
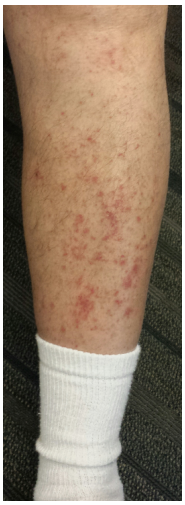
a.



b.



c.

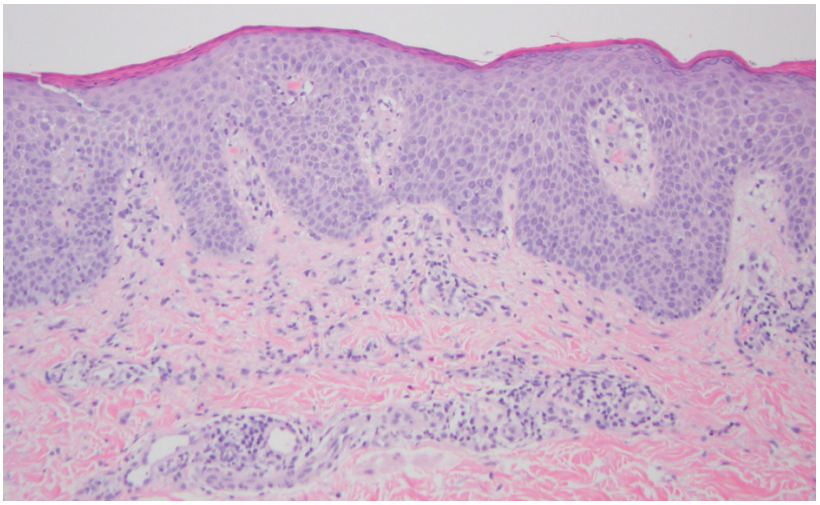


Figure S1. Related to Figure 1. Rash observed in the randomized, double blind, placebo-controlled trial. This occurred in an obese (BMI at baseline: 36.1 kg/m^2) 65-year old male patient diagnosed with diabetes 4 years before baseline. This patient had also been diagnosed with fatty liver disease, hypertension, hyperlipidemia and Barret's esophagus. Rash on **a**) the abdomen and **b**) legs photographed at its peak severity. The rash occurred 29 days from baseline and it was diffuse, pruritic and maculopapular, erupting first at the abdomen and spreading to the patient's chest, axillae, legs and arm. The center of the abdomen was spared. Maximum symptoms occurred at day 3 to 6 after onset, and the patient was prescribed topical steroids (Triamcinolone 0.1% cream) and antihistamines (diphenhydramine 25 mg tablet PRN) to relieve symptoms, but did not have to use these more than four days. The rash improved despite the continuation of the treatment. **(c)** 8 days after the initial symptoms, a punch-biopsy was performed from the left flank, which revealed a perivascular lymphocytic infiltrate and scattered eosinophils, consistent with an eczematous drug eruption. The rash started to resolve 12 days after initial symptoms and was completely abated at day 14 after onset.

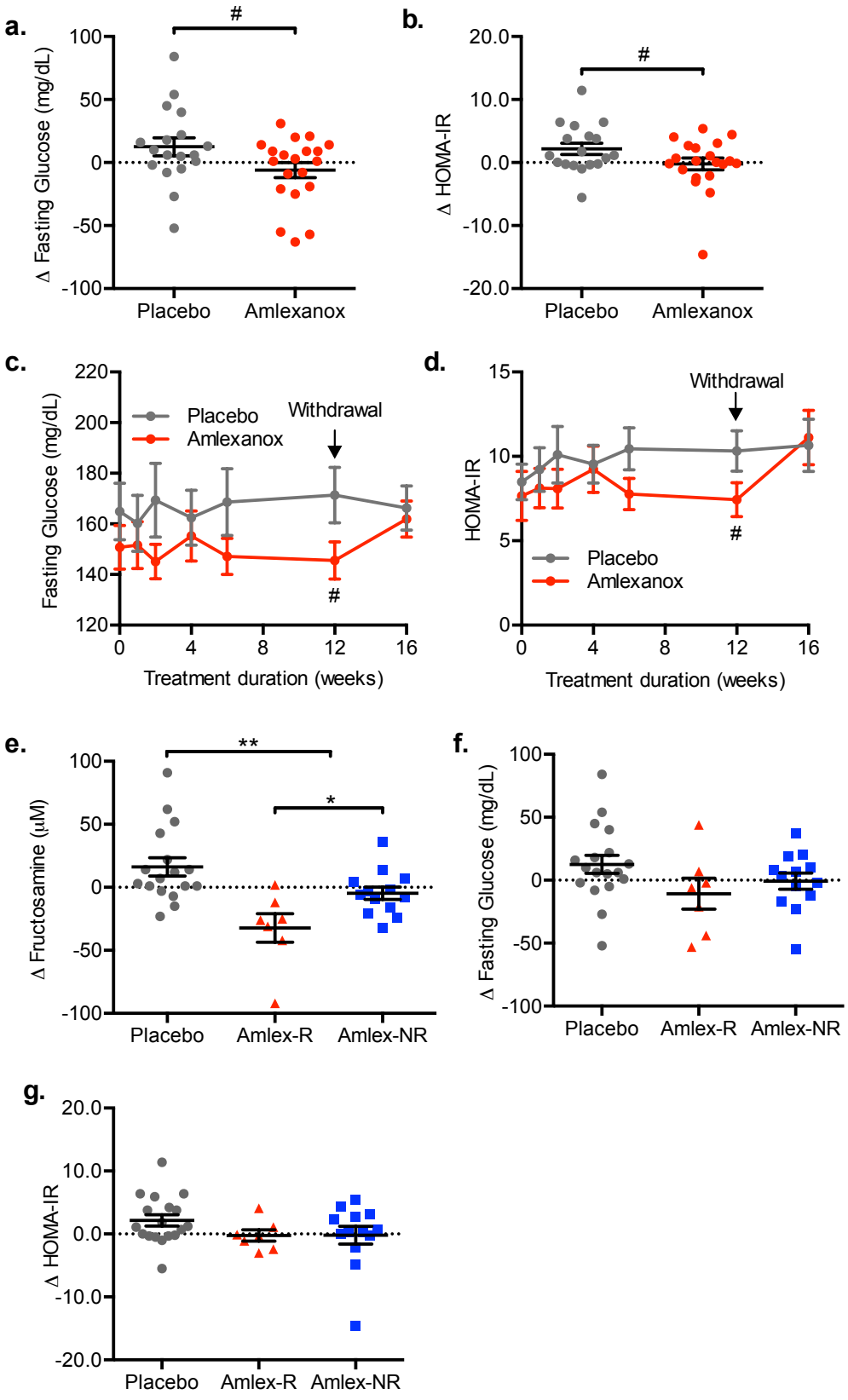

a.
#
100
50
0
-50
-100
Δ Fasting Glucose (mg/dL)
Placebo
Amlexanox
b.
#
20.0
10.0
0.0
-10.0
-20.0
Δ HOMA-IR
Placebo
Amlexanox
c.
220
200
180
160
140
120
Fasting Glucose (mg/dL)
Placebo
Amlexanox
Withdrawal
#
0
4
8
12
16
Treatment duration (weeks)
d.
15
10
5
0
HOMA-IR
Withdrawal
#
Placebo
Amlexanox
0
4
8
12
16
Treatment duration (weeks)
e.
**
*
100
50
0
-50
-100
Δ Fructosamine (μM)
Placebo
Amlex-R
Amlex-NR
f.
100
50
0
-50
-100
Δ Fasting Glucose (mg/dL)
Placebo
Amlex-R
Amlex-NR
g.
20.0
10.0
0.0
-10.0
-20.0
Δ HOMA-IR
Placebo
Amlex-R
Amlex-NR

Figure S2. Related to Figure 3. Effects of amlexanox on fasting glucose and HOMA-IR. a) Change in fasting glucose from baseline to 12 weeks is shown from the entire patient cohort. **b**) Change from baseline to 12 weeks in calculated HOMA-IR from fasting glucose and insulin over the trial period is shown. **c**) Serial fasting glucose levels and (**d**) HOMA-IR across the trial period. **e,f,g**) Difference of fructosamine, fasting glucose and HOMA-IR from baseline with amlexanox treated patients categorized as responders (Amlex-R) and non-responders (Amlex-NR). The responders correspond to the patients with a reduction of HbA1c $\geq$ 0.5 %. n = 18 placebo, 20 amlexanox (7 Amlex-R, 13 Amlex-NR), except **c** and **d** baseline, where n = 21 placebo, 21 amlexanox. * indicates p-value $\leq$ 0.05, # indicates p-value $\leq$ 0.10 (two-tailed t-test). Data shown as individual values and mean ± s.e.m. (**a**, **b**, **e**, **f, g**) or mean ± s.e.m. only (**c**,**d**). HOMA-IR, Homeostatic Model Assessment-Insulin Resistance; NR, non-responder; R, responder.

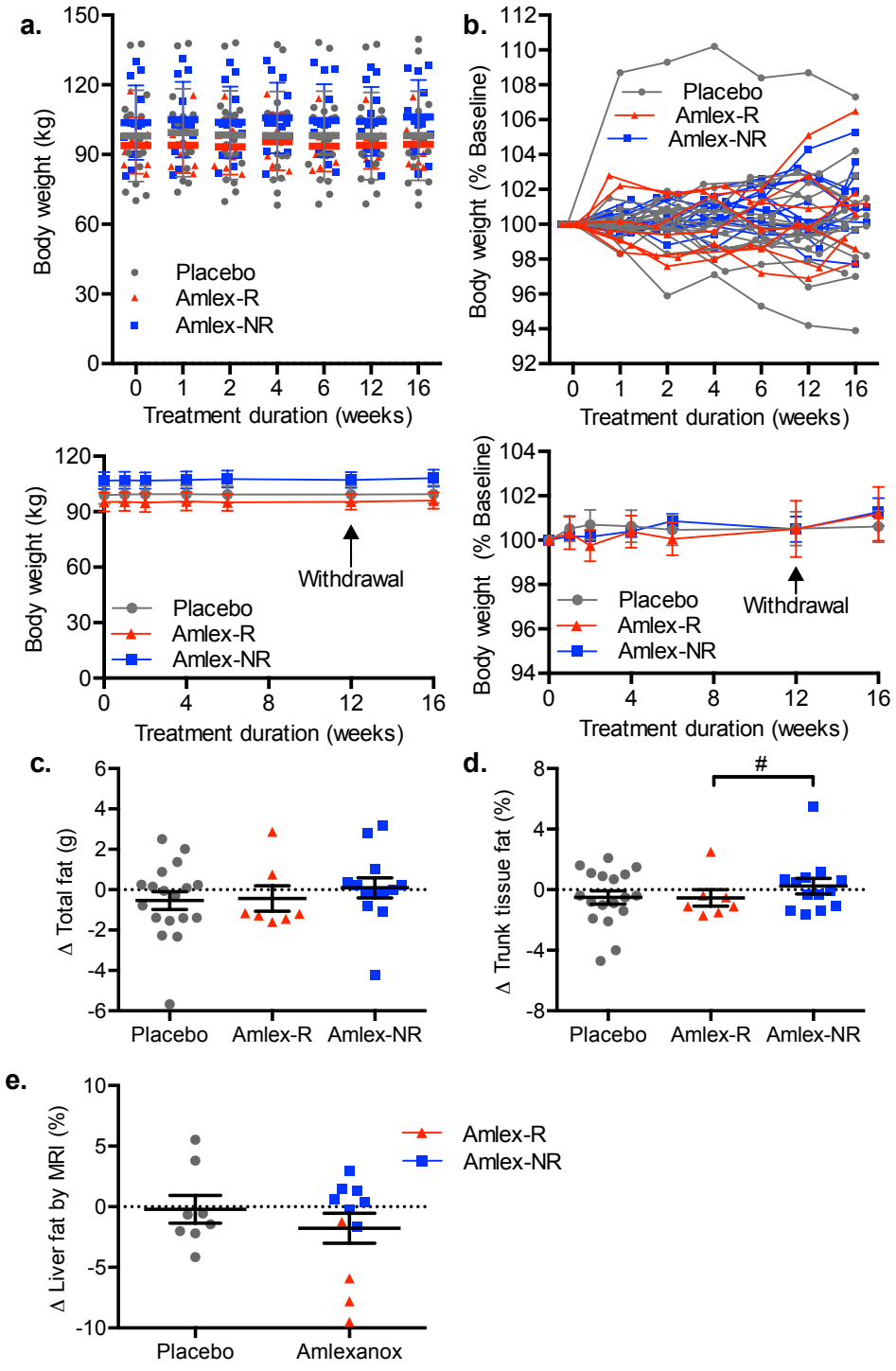


Figure S3. **Related to Figure 3. Amlexanox-induced changes in adiposity. a**) Body weight and **b**) Percent body weight compared to baseline were stable throughout the study. Individual data distribution (upper panels *n* = 18 placebo, 7 amlex-R, 13 amlex-NR) versus mean (lower panels *n* = 16 placebo, 6 amlex-R, 11 amlex-NR) is shown. Mean values contain only complete

data sets as there was missing data in some of the intermediate visits in a few subjects due to the winter storms. **c**) Change in total fat mass percent, (**d**) trunk fat percent and (**e**) liver fat detected by the MRI-Dixon method. # indicates p-value ≤ 0.10 (two-tailed t-test). **c**, **d**) n = 18 placebo, 7 amlex-R, 13 amlex-NR **e**) n = 8 placebo, 4 amlex-R, 7 amlex-NR. Data shown as individual values and mean ± s.e.m, or mean ± s.e.m. only (lower panels **a** and **b**). Amlex-NR, Amlexanox non-responder; Amlex-R, responder.

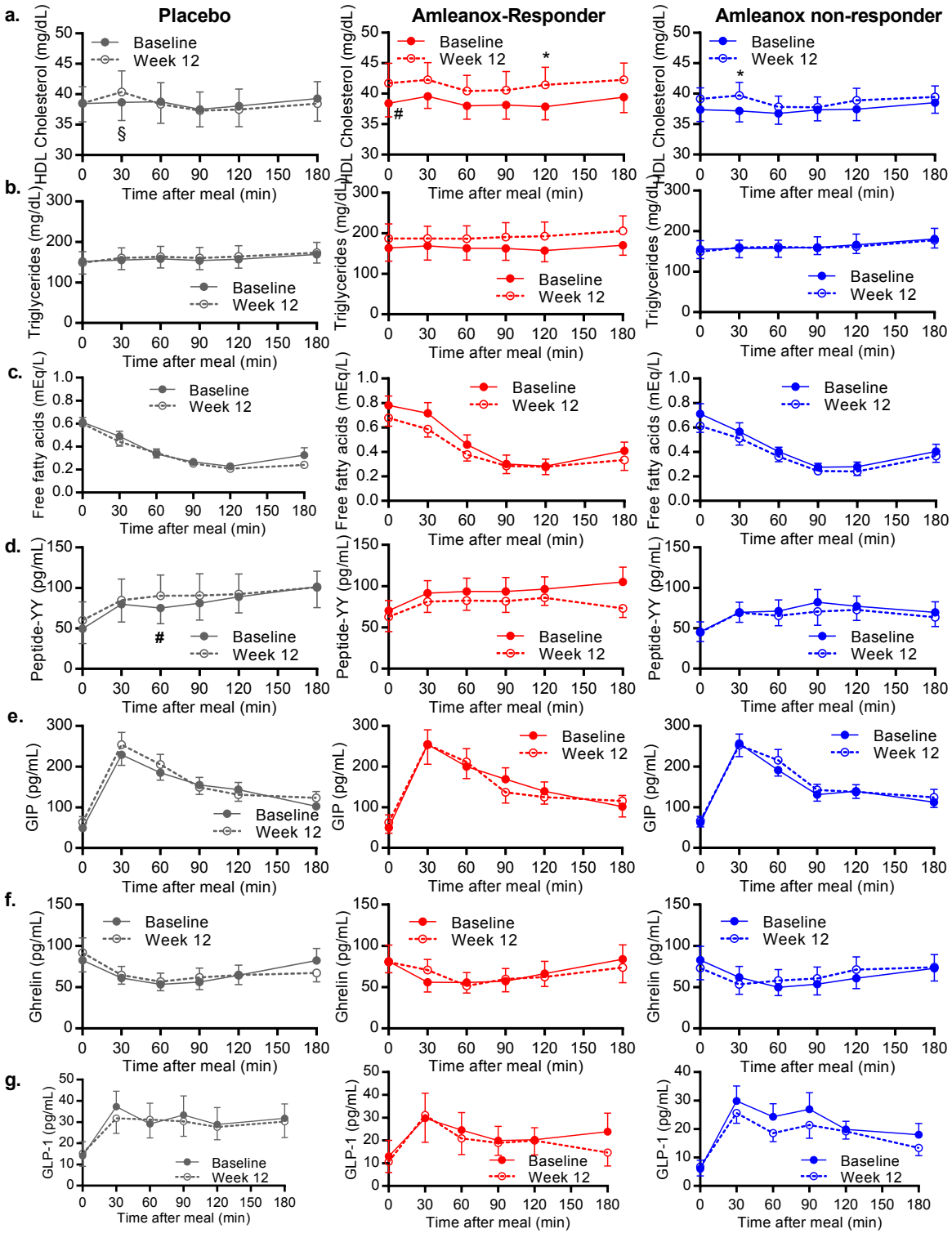
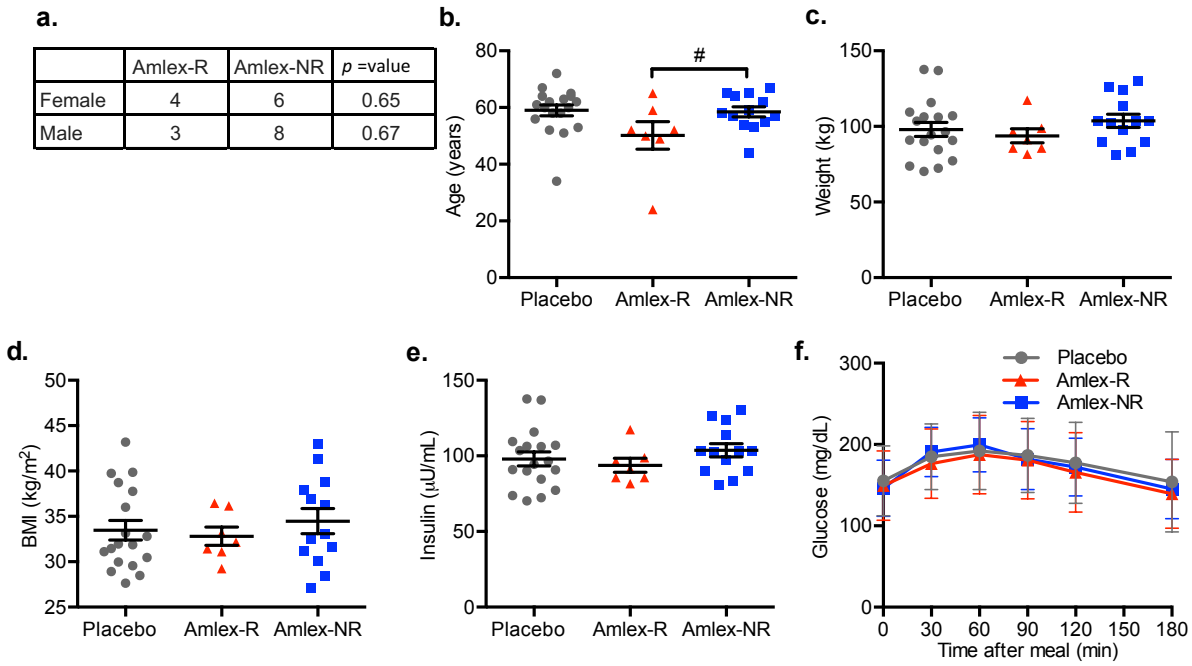


Figure S4. Related to Figure 3. Mixed meal responses at baseline and 12 weeks. Lipid and hormone levels during the mixed meal tolerance test at baseline and 12 weeks: (**a**) HDL cholesterol, (**b**) The triglyceride levels, (**c**) Free fatty acids, (**d**) Peptide YY, (**e**) gastric inhibitory

polypeptide (GIP) (**f**) Grehlin and (**g**) Glucagon-like peptide-1 (GLP-1). *n* = 17 placebo, 7 amlex-R, 13 amlex-NR. * indicates *p*-value ≤ 0.05, # indicates *p*-value ≤ 0.10 (two-tailed paired t-test). Data shown as mean ± s.e.m. Amlex-NR, amlexanox non-responder; Amlex-R, responder.



	Amlex-R	Amlex-NR	*p* =value
Female	4	6	0.65
Male	3	8	0.67

Figure S5. Related to Figure 4. Baseline characteristics in responders and non-responders. **a**) Gender distribution (χ^2 test). **b**) Distribution of age in different groups. **c**) Weight or (**d**) Body mass index (BMI) at baseline did not differ among study groups. **e**) Insulin levels at baseline and (**f**) Post-mixed meal glucose curves at baseline did not differ among the study groups. Amlex-NR, Amlexanox non-responder; Amlex-R, responder. *n* = 18 placebo, 7 amlex-R, 13 amlex-NR. * indicates *p*-value ≤ 0.05, # indicates *p*-value ≤ 0.10 (two-tailed t-test). Data shown as individual values and mean ± s.e.m (**b-e**), or mean ± s.e.m. only (**f**). Amlex-R: responder, Amlex-NR: Amlexanox non-responder, BMI: body mass index.

Table S1: Related to Figure 1. Treatment emergent adverse events in the open-labeled trial.

Adverse Event	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**
Rash	X			X		
Polyuria					X	
Nausea					X	
Restless Legs		X				
Upper respiratory infection		X		X		
Elevated Triglycerides	X					
Breast Abscess			X			
Subcutaneous nodule at biopsy site						X

All events were graded mild to moderate. There were no serious adverse events.

Table S2: Related to Figure 3. Subject disposition for key study parameters.

Parameter	Placebo *n included / n excluded, reasons*	Amlexanox *n included / n excluded, reasons*
HbA1c	18/3 Two early drop-out, One withdrawn for high HbA1c after Day 1	20/1 One withdrawn without dosing due to high CK
Fructosamine	17/4 One additional excluded for missing baseline value due to order entry error	20/1
Biochemical parameters*	18/3	20/1
CRP	17/4 One additional excluded for compromised baseline sample	20/1
Mixed meal test	18/3	20/1
DEXA	18/3	20/1
MRI for liver fat	8/13 Twelve not completed for funding reasons, one with baseline study, 12-week not done: SAE	11/10 Ten not completed for funding reasons
Fat Biopsy	16/5 One 12-week not done due to SAE and pne subject aborted baseline biopsy	20/1
16 week follow-up	18/3	19/2 One patient did not return for 16-week visit

*24-hour urine protein samples were available from only 16 patients in each group due to patient compliance.

Table S3: Related to Figure 3. Treatment-emergent adverse events in the randomized, double blind, placebo-controlled study.

Event Terms	Placebo	Amlexanox	P-value
Rash[a]	3	4	0.70
Gastro-intestinal (nausea, diarrhea, abdominal pain)	7	4	0.37
Infection (gastroenteritis, sinusitis, upper respiratory, paronychia)	9	7	0.62
Neurological (headache, worsening neuropathy, lightheadedness, vertigo)	5	4	0.74
Cardiovascular			
NSTEMI[b]	1	0	0.32
Increased blood pressure	1	0	0.32
Palpitations	0	1	0.32
Other			
Myalgias	2	2	1.00
Pruritis (no rash)	0	2	0.16
Urinary frequency	0	1	0.32
Hot flushes	1	0	0.32
Lower extremity edema	0	2	0.16
Elevated CK	0	1	0.32
Nephrolithiasis	0	1	0.32
Hypoglycemia	1	0	0.32

[a]*Graded as possibly-to definitely related to treatment.*
[b]*Graded as serious adverse event, caused premature un-blinding.*

Table S4: Related to Figure 3. Key data from individual patients.

Patient Number	Group	Response Status	Disposition	HbA1c Baseline %	HbA1c 12weeks %	Fructosamine Baseline µmol/L	Fructosamine 12-weeks µmol/L	Liver Fat MRI %	Liver Fat MRI %
AMLX2-01	A	R	Completed	7.4	6.5	209	211	17.9	10.1
AMLX2-02	A	R	Completed	7.1	6.5	252	240	14.2	4.7
AMLX2-03	P	N/A	Completed	7.1	7	239	240	27	25
AMLX2-04	P	N/A	Completed	9.9	10.5	441	463	15.4	13.2
AMLX2-05	A	NR	Completed	6.7	6.4	209	203		

AMLX2-06	A	NR	Completed	7.3	7.4	281	260	1.7	1.45
AMLX2-07	P	N/A	Completed	6.9	6.6	299	300	9.1	4.94
AMLX2-08	P	N/A	Completed	8	10.4	310	372	5.5	4.93
AMLX2-09	A	NR	Completed	6.9	7	263	255	16.92	18.4
AMLX2-10	P	N/A	Completed	7	7	259	273		
AMLX2-11	A	NR	Completed	6.9	7.1	211	247	22.9	23.27
AMLX2-12	A	NR	Completed	6.8	6.8	248	246	10.44	13.4
AMLX2-13	P	N/A	Completed	8.3	9.1	262	353	18.26	23.78
AMLX2-14	P	N/A	Completed	7.3	7.4	252	255	19.9	18.46
AMLX2-15	P	N/A	Completed	7.3	7.6	244	258		
AMLX2-16	P	N/A	Completed	6.7	6.6	213	214	4.1	3.44
AMLX2-17	A	NR	Completed	6.7	7.1	245	249		
AMLX2-18	P	N/A	Completed	7	7	276	319	10.65	
AMLX2-19	A	R	Completed	9.5	7.1	366	274	24.84	23.6
AMLX2-20	A	NR	Completed	8.4	8.6	309	323	31.35	32.7
AMLX2-21	A	NR	Completed	6.7	7.2	241	232	15.23	13.6
AMLX2-22	P	N/A	Completed	7.4	8.1	291	343		
AMLX2-23	P	N/A	Completed	8	7.6	266	251	5.49	9.3
AMLX2-24	A	R	Completed	8	7.5	271	246	33.9	28
AMLX2-25	A	N/A	Completed	10.1	10.2	402	409	8.62	9.2
AMLX2-26	P	N/A	Early drop-out	7.8		292			
AMLX2-27	P	N/A	Early-drop-out	10.9		439			
AMLX2-28	P	N/A	Completed	6.6	6.8	258	$		
AMLX2-29	P	N/A	Completed	6.6	6.4	236	229		
AMLX2-30	A	NR	Completed	7.4	8.1	325	301		
AMLX2-31	A	R	Completed	8.2	7.2	316	274		
AMLX2-32	P	N/A	Completed	8.3	8.1	294	271		
AMLX2-33	A	NR	Completed	7	6.8	251	235		
AMLX2-34	P	N/A	Completed	7.7	7.7	258	270		
AMLX2-35	P	N/A	Early drop-out	8.2		270			
AMLX2-36	A	N/A	Early drop-out	7.4		240			
AMLX2-37	P	N/A, *	Completed	7.6	6.9	234	231		
AMLX2-38	A	NR	Completed, no week 16	8.5	8.1	342	310		
AMLX2-39	A	R	Completed	8.6	7.8	289	258		
AMLX2-40	A	NR	Completed	8.6	8.8	321	318		
AMLX2-41	P	N/A	Completed	8.6	8.9	263	270		
AMLX2-42	A	R	Completed	7	6.5	253	227		

A: Amlexanox, P: Placebo, R: Responder in the Amlexanox group, NR: Non-responder in the Amlexanox group, N/A: Not applicable to be classified as a responder, either early drop-out or placebo group

$ 12-week fructosamine missing due to order entry error

*Placebo effect: Clinically significant drop in HbA1c in the placebo group

Note: MRI was attempted in 20 subjects only until 20 baseline MRIs were completed, not done in patients 5, 10, 15, and 22 due to presence of a contraindication for an MRI or history of claustrophobia

Table S5: Related to Figure 4. Amlexanox and amlexanox metabolites in serum. Spreadsheet in separate attachment.

Table S6: Related to Figure 5. Pathway enrichment of genes whose expression was either upregulated or downregulated by amlexanox treatment in the responders.

Upregulated			
Term	**Count**	***p*-value**	**Genes**
Endocytosis	20	0.00	ARFGAP1, HRAS, CLTB, CHMP6, AP2S1, VPS37B, PIP5K1C, ADRBK1, VPS37D, FAM125A, RAB11FIP3, AP2A1, RAB11B, VPS24, HSPA7, HGS, VPS28, EPN1, ARAP1, SH3GL1
Notch signaling pathway	8	0.01	CTBP1, NOTCH1, JAG2, DLL1, RFNG, LFNG, NCOR2, DVL1
Ribosome	11	0.01	RPS28, RPL18A, RPL13, RPL8, RPLP1, RPS15, RPLP2, RPL36, RPS9, FAU, RPL28
Axon guidance	14	0.01	HRAS, PLXNA1, LIMK1, PLXNB2, EFNB1, EPHB3, EPHB6, SEMA6C, RAC3, SEMA3F, PAK4, SEMA3B, ROBO3, RHOD
VEGF signaling pathway	10	0.01	HRAS, SPHK2, MAP2K2, RAC3, PLA2G6, HSPB1, BAD, SHC2, PIK3R2, AKT2
Huntington's disease	17	0.01	ATP5D, CLTB, POLR2E, POLR2L, NDUFB7, AP2S1, POLR2J, COX8A, CYC1, COX5B, NDUFS7, GPX1, PLCB3, UQCR11, AP2A1, BBC3, NDUFS8
Acute myeloid leukemia	8	0.02	CEBPA, JUP, HRAS, MAP2K2, RPS6KB2, BAD, PIK3R2, AKT2
Lysosome	12	0.03	ATP6V0C, TCIRG1, NAGLU, NAGPA, CLTB, ARSA, GAA, CTSD, ABCA2, MAN2B1, IDUA, CTSF
Oxidative phosphorylation	12	0.05	ATP5D, ATP6V0C, TCIRG1, NDUFS7, ATP6V0E2, UQCR11, NDUFB7, NDUFS8, CYC1, COX8A, COX5B, NDUFA11
Arachidonic acid metabolism	7	0.05	GGT5, GPX1, PTGES2, PTGDS, GPX4, PLA2G6, LTC4S
ErbB signaling pathway	9	0.06	HRAS, MAP2K2, PAK4, RPS6KB2, BAD, MAP2K7, SHC2, PIK3R2, AKT2
MAPK signaling pathway	20	0.06	TRAF2, HRAS, TAOK2, MAP2K2, MKNK2, ECSIT, TGFB1, RPS6KA4, RAC3, GADD45G, MAPK8IP3, HSPA7, RRAS, HSPB1, PLA2G6, MAPK8IP1, MAP2K7, CD14, AKT2, MAP3K11
Insulin signaling pathway	12	0.07	SREBF1, HRAS, MAP2K2, MKNK2, FASN, RPS6KB2, IGF2, BAD, SHC2, LIPE, PIK3R2, AKT2
Phosphatidylinositol signaling system	8	0.07	PLCB3, CDIPT, DGKQ, INPPL1, PLCD3, DGKZ, PIP5K1C, PIK3R2
Chronic myeloid leukemia	8	0.07	HRAS, CTBP1, MAP2K2, BAD, SHC2, TGFB1, PIK3R2, AKT2
Downregulated			
Term	**Count**	***p*-value**	**Genes**
Spliceosome	4	0.01	HNRNPA3, THOC2, HSPA8, PRPF38A
Biosynthesis of unsaturated fatty acids	2	0.06	FADS1, ELOVL6

Table S7: Related to Figure 5. Q-PCR primer sequences.

Gene	Common protein name	Primers
ADIPOQ	Adiponectin	F 5'-GCAGGCATCCCAGGACATC-3' R 5'-GCGATACATATAAGCGGCTTCT-3'
ADRB1	β-adrenergic receptor 1	F 5'-ATCGAGACCCTGTGTGTCATT-3' R 5'-GTAGAAGGAGACTACGGACGA-3'
ADRB2	β-adrenergic receptor 2	F 5'-TGGTGTGGATTGTGTCAGGC-3' R 5'-GGCTTGGTTCGTGAAGAAGTC-3'
ADRB3	β-adrenergic receptor 3	F 5'-GACCAACGTGTTCGTGACTTC-3' R 5'-GCACAGGGTTTCGATGCTG-3'
CCL2	Monocyte Chemotactic Protein 1	F 5'-CAGCCAGATGCAATCAATGCC-3' R 5'-TGGAATCCTGAACCCACTTCT-3'
COX5B	Cytochrome C Oxidase Subunit 5B	F 5'- TGTGAAGAGGACAATACCAGCG-3' R 5'- CCAGCTTGTAATGGGCTCCAC-3'
DIO2	Deiodinase, Iodothyronine, Type II	F 5'-TCCAGTGTGGTGCATGTCTC-3' R 5'-CTGGCTCGTGAAAGGAGGTC-3'
ELOVL3	ELOVL Fatty Acid Elongase 3	F 5'-TGGGGCATTATGGGGACTGT-3' R 5'-AGGACCAGAATTTGACTGTGGA-3'
FASN	Fatty Acid Synthase	F 5'-CCGAGACACTCGTGGGCTA-3' R 5'-CTTCAGCAGGACATTGATGCC-3'
FGF21	Fibroblast growth factor 21	F 5'-GCCTTGAAGCCGGGAGTTATT-3' R 5'-GTGGAGCGATCCATACAGGG-3'
IL4	Interleukin 4	F 5'-CGGCAACTTTGTCCACGGA-3' R 5'-TCTGTTACGGTCAACTCGGTG-3'
LIPE	Hormone-Sensitive Lipase	F 5'-AGGAGCCAGCATTGAGACAAA-3' R 5'- CGCAGGTGTTGATTCAGCTTC-3'
PPARGC1A	PPARγ Coactivator 1-α	F 5'-TGAAGACGGATTGCCCTCATT-3' R 5'-GCTGGTGCCAGTAAGAGCTT-3'
PPARGC1B	PPARγ Coactivator 1-β	F 5'-GATGCCAGCGACTTTGACTC-3' R 5'-ACCCACGTCATCTTCAGGGA-3'
PRDM16	PR/SET Domain 16	F 5'-GTTCTGCGTGGATGCAAATCA-3' R 5'-GGTGAGGTTCTGGTCATCGC-3'
RPLP0	Ribosomal Protein, Large, P0	F 5'-GCAGCATCTACAACCCTGAAG-3' R 5'-CACTGGCAACATTGCGGAC-3'
UCP1	Uncoupling Protein 1	F 5'-AGGTCCAAGGTGAATGCCC-3' R 5'-TTACCACAGCGGTGATTGTTC-3'
UCP2	Uncoupling Protein 2	F 5'-GGAGGTGGTCGGAGATACCAA-3' R 5'-ACAATGGCATTACGAGCAACAT-3'
UCP3	Uncoupling Protein 3	F 5'-AAGGTCCGATTTCAGGCCAG-3' R 5'-GCGATGGTTCTGTAGGCGTC-3'